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THE MICROSCOPICAL EXAMINATION
OF
FOODS AND DRUGS

BY THE SAME AUTHOR.

AN INTRODUCTION TO THE STUDY
OF MATERIA MEDICA.

Designed for Students of Pharmacy and Medicine.

With 213 Illustrations. 15s.

THE
MICROSCOPICAL EXAMINATION
OF
FOODS AND DRUGS

A PRACTICAL INTRODUCTION TO THE
METHODS ADOPTED IN THE MICROSCOPICAL EXAMINATION
OF FOODS AND DRUGS, IN THE ENTIRE, CRUSHED
AND POWDERED STATES

BY

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WITH 168 ILLUSTRATIONS



LONDON
J. & A. CHURCHILL
7 GREAT MARLBOROUGH STREET

1903

PRINTED BY
SPOTTISWOODE AND CO. LTD., NEW-STREET SQUARE
LONDON

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PREFACE

NEARLY half a century has elapsed since Dr. Arthur Hill Hassall, in his classical works on the detection of adulterations in food and medicine, strongly advocated the use of the microscope as a valuable aid to chemical analysis in the examination of a large variety of vegetable substances, an advocacy that he supported by the publication of a great number of analyses.

During this time considerable progress has been made in the detection of such adulteration by chemical analysis, and numerous valuable memoirs and excellent text-books replete with details and literary references have been published. That branch of the subject, however, to which Dr. Hassall particularly devoted his attention—viz. the examination by the microscope—has unfortunately quite failed to keep pace with the examination by chemical analysis. Such portions of the present manuals of analysis as are devoted to the use of the microscope provide instructions that are but meagre, descriptions that lack precision, and illustrations that are wanting in detail. And yet the microscope is capable of furnishing, with the expenditure of a minimum of material and also often of a minimum of time, information concerning the substances analysed that cannot be obtained by any other means. With much truth Hassall observes that ‘applying the microscope to food, it appears that there is scarcely a vegetable article of consumption, not a liquid, which may not be distinguished by means of that instrument. Further, that all those adulterations of these articles which consist in the addition of other vegetable sub-

stances, and which constitute by far the majority of the adulterations practised, may likewise be discovered and discriminated by the same means.¹

Since the publication of Hassall's works much has been done by the State to protect the public from the frauds that were then frequently practised upon it. There exist at the present time a large number of analysts engaged, more or less regularly, in the examination of foods and drugs. To these in particular the ability to use the microscope for the purpose of detecting adulteration or confirming results arrived at by other means must be of paramount importance. Indeed it is difficult to understand how any public analyst that is unable to make an expert use of the microscope can be competent to discharge his duties. Upon this point the Local Government Board, the authority entrusted with the framing of regulations regarding the appointment of public analysts, has pronounced a definite opinion by making competency in the use of the microscope a necessary qualification, and the Institute of Chemistry of Great Britain and Ireland has established an examination that conforms in this respect with the requirements of the Board.

The Pharmaceutical Society of Great Britain has also recognised the importance to the pharmacist of skill in the use of the microscope for identifying the nature and determining the quality of powdered drugs. It is now some years since the Society introduced into its curriculum of study a suitable course of training in the use of the microscope, and into its examinations a test of the ability to use the knowledge thus acquired. Investigations recently conducted by the Society at the request of the General Medical Council have also shown that the microscopical examination of powdered drugs yields results of far greater value in determining their identity and purity than such chemical data as the amount of ash yielded by them.

Before the microscopical examination of vegetable foods

¹ *Adulterations Detected*, 1857, p. 45.

and drugs can be intelligently practised, a general knowledge of botany and a fairly sound and thorough acquaintance with botanical histology are absolutely necessary. It is as impossible for anyone to become a competent microscopist without such preliminary knowledge, as to become a competent analytical chemist without first acquiring a sound knowledge of the theory and principles of the science of chemistry. For this reason it appears to me that the training now given in the School of Pharmacy of the Pharmaceutical Society—comprising, as it does, a knowledge of the principles upon which the sciences of botany and chemistry rest as well as training in the application of the knowledge thus acquired, is admirably adapted to fit a man to become an expert not only in the microscopical but also in the chemical examination of foods and drugs.

While there exist a number of excellent text-books of botany to aid the student in acquiring a knowledge of vegetable histology, there does not exist an English text-book specially devoted to practical instruction in the methods of examining vegetable foods, drugs, and their powders. The botanical text-books are not suitable for this purpose. They deal with the structure of vegetable organs from a general point of view, and devote little or no attention to the details that distinguish the individual members of any one class from one another, nor are the methods they employ generally suitable for analytical purposes. It was with a view to supplying this want that I undertook the compilation of the present volume. In it I have endeavoured to introduce the student to the chief methods adopted in the examination of vegetable foods and drugs, entire, crushed, and finely powdered. The best, in fact the only suitable, means of doing this seemed to me by selecting certain typical examples and describing the means by which they may be examined. The well-known danger that is incurred in teaching by types I have endeavoured to avoid by including a number of other examples which I have treated more briefly ; hence in the earlier sections, dealing with starches,

stems, and leaves, a comparatively large number have been examined, or at least referred to, while in the succeeding sections the number has been less. The selection of the substances to be examined has been the subject of grave consideration, inasmuch as it appeared desirable to examine such as illustrated varying methods, offered important but varying features in their structure, and yet were of general interest and in more or less common use.

The subject matter has been divided into twelve sections, which have been so arranged that the student may begin with the simplest and proceed to the most complex, acquiring, as he proceeds, a knowledge of various tests and operations that are of more or less general use. Thus I have commenced with the starches, which require but little preparation, and have proceeded to the fruits, which commonly possess a complex structure, and to the roots, which present difficulties in their identification when powdered. In order to exhibit the great variety of forms that starch may assume I have described and illustrated a rather large number, including, for practical reasons, all the most important. In dealing with leaves I have selected bearberry as an example of a coriaceous leaf that requires soaking in water before it is cut, that is easily cut, and that allows of the epidermis being separated after digestion with caustic potash; senna, as a type of papery leaves that are best softened by exposure to a moist atmosphere, that are best cut in packets, and that exhibit their epidermis after soaking in chloral hydrate; tea, on account of its importance as well as on account of the remarkable sclerenchymatous idioblasts it contains; buchu, because it contains mucilage, oil glands, and hesperidin; belladonna, as an example of a solanaceous leaf with bicollateral bundles and sandy crystals; henbane and stramonium, as important objects for comparison, and so on throughout all the substances examined.

In very few instances is the examination of any food or drug complete without the preparation of sections in various directions, the separation of the tissues by suitable means, and

the examination of the powder ; but nevertheless I have not dealt with all these operations until senna leaves are reached because experience has taught me that the student may at that point most advantageously commence the study of the powder. For this purpose senna leaves of known genuineness should be reduced to a powder that will pass through a No. 60 sieve ; this will be coarser than the powdered senna of commerce, and more easily examined.

Although I have endeavoured to deal with substances yielding important powders, I have refrained from any attempt to embrace all such as the analyst or pharmacist may be likely to meet with. To supply this latter want I have in conjunction with M. Eugène Collin published a series of memoirs in the ' Pharmaceutical Journal ' which will shortly be reprinted in the form of an Anatomical Atlas. Such an atlas can, however, be intelligently used only by those who have had preliminary training in botanical histology and in the methods of examining drugs and powders. The present volume is intended to afford to the student who has already been grounded in botany the instruction and advice necessary to enable him to undertake the examination of vegetable powders. In its preparation I have received much valuable assistance from Mr. C. Heslop, Demonstrator in Pharmaceutics (1901-2), and Mr. T. E. Wallis, Assistant Lecturer and Senior Demonstrator in Chemistry and Physics. To Mr. Heslop I am indebted for help in reproducing several of the illustrations, while figs. 56, 116, 117, 118, 119, and 161 were prepared from drawings made by Mr. Wallis during the examination of the several substances under my own supervision. I have also made full use of the literature of the subject, particularly of the publications of Meyer, Moeller, Schimper, Tschirch, Tschirch and Oesterle, and Vogl.

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INTRODUCTION

To the student who accepts this work as a guide to conduct him from a knowledge of the general structure of vegetable organs to the study of the anatomical details of foods, drugs, and their powders, I address the following observations.

In order to thoroughly understand the anatomy of any drug, it is necessary first to examine it with a lens, then to cut sections in different directions and through different parts ; to disintegrate the tissues by suitable means, and compare these with the tissues observed in the sections ; to examine the powdered drug and compare the tissues, cells, and cell contents observed with those that previous examination has disclosed, noting the changes that they have undergone and seeking and utilising special reactions to render more conspicuous such as are not easily detected.

Most strongly must I insist upon the necessity of recording observations by means of sketches. This affords a most valuable training for the powers of observation, for that which has not been accurately seen cannot be satisfactorily sketched, and conversely that which the student cannot sketch has not been properly seen or understood. Much time may, however, be lost by injudicious or indiscriminate sketching. Diagrammatic sketches under a low power should first be made with a view of indicating the positions and extents of the various tissues ; of such diagrammatic sketches figs. 54, 72, and 84 may serve as examples.

The details of these tissues should then be drawn on a scale large enough to allow of the necessary minutiae being

introduced. Figs. 61, 116 to 119, 141, 142, and 143 are examples of such. All sketches should be drawn in pencil and corrected until accurate, for one cell accurately drawn is a valuable record, whereas a hundred cells inaccurately drawn can only mislead. A little difficulty may at first be experienced in discriminating between the important and the unimportant; I have therefore in a number of instances endeavoured to aid the student by indicating what should be sketched.

It may of course be perfectly possible, by means of atlases, descriptions, or keys, to identify an unknown powder, but the identity cannot be considered as satisfactorily established until the powder has been compared with the powder of the drug with which it has been identified.

The student is recommended to prepare his own powders from the entire drug, passing them through a No. 60 or No. 80 sieve. After these have been studied the finer commercial powders which present more difficulty may be examined.

Finally, let me impress upon him the fact that there is no royal road available. Facility in the microscopical examination of foods and drugs can be acquired only by study and practice; without these it is impossible to become an expert in the use of the microscope.

FOODS AND DRUGS

SECTION I

STARCH

INTRODUCTION

STARCH is one of the most widely distributed of the cell contents of plants. It is found in all classes, with the exception of the Fungi, and is met with in different parts of the same plant. It may, under certain circumstances, be detected in the form of minute grains in the chloroplastids of the leaf or stem, from which organs it is transported, in soluble form, to other organs destined to receive it. In these it may either be temporarily deposited until required for the growth of particular parts of those organs, as is the case with the small starch grains deposited in the epidermis of the linseed and other seeds which are converted into mucilage, or it may be more permanently deposited in comparatively large quantity as a reserve material to supply the subsequent needs of the plant. Seeds, fruits, rhizomes, roots, and aërial stems form the principal reservoirs for the storage of reserve starch, the presence of which often renders them valuable as food stuffs or as sources of commercial starch.

Careful examination has shown that the starch grains produced by a particular plant are remarkably constant in size, shape, and general characters, but it has also been shown that the starch grains of one plant often differ from those of other plants to such an extent as to render the two kinds easily distinguishable. Sometimes, too, the starch grains produced by the various species of a single genus, or natural order, exhibit a remarkable

general resemblance to one another, as is the case, for instance, with the starch of many species of Leguminosæ.

The careful study of the starch grains, and especially of those deposited as reserve starch, becomes therefore of primary importance both as a means of identifying the source of the different commercial varieties of starch and of distinguishing various starch-containing drugs from one another. The detection of starch is also frequently of great value as constituting a means of determining the adulteration of the powder of a drug naturally free from starch with either starch itself or with a drug containing that substance.

The following, therefore, are the chief points to be borne in mind in the examination of starch :

- (1) The means by which starch grains can be identified as such.
- (2) The means by which the starches of different plants may be distinguished from one another.

Examination of Potato Starch

Mounting.—Put a small drop of water on a slide ; take a little potato starch on the point of a knife and transfer it to the water ; mix thoroughly with a mounted needle and carefully cover with a coverslip. This should be done by gradually lowering the coverslip by means of the needle, preventing it from slipping by holding the finger against the edge that rests upon the slide. Care should be taken to avoid undue pressure, which is liable to crush the grains. Both slide and coverslip must be scrupulously clean, and any excess of water should be removed by a strip of filter paper, which for this purpose is preferable to blotting paper. It is very desirable to take in the first instance a drop of water of about the right size, but this can be attained only by practice.

Strict cleanliness in the mounting of objects for microscopic examination cannot be too strongly insisted upon. Excess of the mounting medium, if not removed as directed, allows of the coverslip floating and of the objects under examination moving ; moreover, the liquid is very liable to find its way on to the upper surface of the coverslip and thence on to the front lens of the objective, in either case obscuring clear vision.

Too much starch is also objectionable, as the grains then lie over one another and accurate observation becomes impossible.

The presence of one or two bubbles of air is not a matter of importance, but a large number of them should be avoided; if they have by accident found their way into the preparation, as they may do if the coverslip has not been carefully lowered, a fresh preparation should be made.

Shape.—Examine the slide first with a low power ($\frac{2}{3}$ inch or $\frac{1}{2}$ inch) and then with a high power ($\frac{1}{8}$ inch). The starch consists of grains of variable size. They have an oval, ovate, or ellipsoidal outline; some are triangular or even obscurely quadrangular, with rounded angles, and resemble oyster shells in shape.



FIG. 1.—Potato Starch. $\times 240$. (Greenish and Collin.)

The outline alone, however, does not give a sufficient clue to the shape. This must be ascertained by making the grains roll so that the same grain may be viewed in different positions. Examine a slide under the low power, and while it is under observation gently touch the edge of the coverslip with a needle. This will usually produce sufficient movement to cause some of the grains to roll and thus exhibit their form. In the case of small starch grains sufficient movement may be produced by bringing a drop of alcohol on the slide to the edge of the coverslip; the alcohol as it mixes with the water sets up currents that carry the grains with them. Potato starch, when made to roll, is seen to be distinctly, though not strongly, flattened.

Observe here and there a starch grain composed of two grains adhering by their broader (flattened) ends ; such grains are termed 'compound.' Sometimes a compound grain is subsequently entirely surrounded by concentric rings of starch material ; it is then called a 'semi-compound' grain.

Hilum.—Near one extremity (usually the narrower) there is a point around which concentric lines are arranged. This is the hilum. It appears either as a dark spot (high focus) or as a bright spot of distinctly reddish colour (low focus) ; this colour is apparent only. In some of the grains there is a small linear or V-shaped fissure through the hilum ; this is often, but incorrectly, spoken of as the hilum—it is a fissure produced by the shrinkage attendant upon the drying of the grain.

Note that in potato starch the hilum is not in the centre of the grain ; it is eccentric. It is often desirable to indicate the exact position of an eccentric hilum ; this is done by measuring the distance between it and the nearer as well as the further margin of the grain and stating the ratio between these figures, which expresses the degree of eccentricity, as a fraction. Thus in potato starch the greater distance is about five times the lesser, and the eccentricity is therefore about $\frac{1}{5}$.

Striations.—Surrounding the hilum are a number of fine concentric lines ; these are termed *striations* or *striæ*. They are said to be caused by variations in the amount of moisture present, the dark striations being comparatively rich, while the colourless intervening portions are comparatively poor in moisture ; Meyer ascribes them to differences in the minute crystalline particles of which he believes the starch grain to consist. Observe that, like the hilum, the striations appear dark at high focus but bright and reddish-coloured at low focus. Observe also that in potato starch there occur at intervals striations that are more strongly marked than the others.

The grains of many varieties of starch do not exhibit any striations at all ; few exhibit them so conspicuously as potato starch does, and still fewer show some more and others less strongly marked.

Size.—The size of a starch grain and of other microscopic objects is usually ascertained by measurement with an ocular micrometer.

This instrument consists of a small glass circle on which an

arbitrary scale is engraved. In the ordinary form this scale contains ten divisions, each of which is subdivided into ten subdivisions. The micrometer is introduced into the eyepiece by unscrewing the eye-lens and dropping it upon the diaphragm, the position of which has to be adjusted so that the scale on the micrometer appears in focus on looking through the eyepiece. It is convenient to keep an eyepiece with the micrometer fixed in it, ready for measuring an object at any moment.

The value of each division or subdivision of this scale has to be ascertained by means of a millimetre scale.

The millimetre scale in its ordinary form consists of a centimetre engraved upon a glass slide and divided into ten millimetres; one of the latter is subdivided into ten parts. The slide is focussed in the usual way, the eyepiece, however, being used that contains the micrometer scale; the former must be rotated until the two scales are exactly superposed, when the number of divisions that correspond to, say, 1 millimetre on the millimetre scale can be read off. Thus, supposing 1 millimetre be exactly covered by 95 subdivisions of the ocular micrometer, then each subdivision of the latter will indicate $\frac{1}{95}$ or 0.0105 millimetre.

It is, however, usual to express measurements in terms, not of a millimetre, but of a micron (μ). A micron is the one-thousandth part of a millimetre, and consequently in the example quoted each subdivision of the scale will indicate 10.5 micra.

The value of the subdivisions of the micrometer will, of course, vary with the eyepiece and objective used. It will be found convenient to retain an eyepiece specially for measuring and to determine and record the value of the scale for each objective in general use.

Having determined the value of the ocular micrometer scale as described, remove the slide with the millimetre scale from the stage of the microscope and substitute for it the slide with potato starch grains. Bring the grain to be measured nearly into the centre of the field, and then rotate the eyepiece until the micrometer scale coincides with the long axis of the grain. The number of subdivisions the grain covers can then be easily read off and converted into micra by multiplying by the previously ascertained factor.

In measuring starch grains in this way it is customary to

measure them in water and to neglect the slight swelling that takes place when the dry grain is mounted in that liquid. In addition to the largest and smallest grain those of most frequent occurrence should also be measured. It is sometimes desirable to ascertain the width as well as the length, but this is not often necessary.

Sketching.—Having thus carefully examined the starch, record the results by sketching a few of the grains. The importance of this as a means of training the power of observation and ensuring that no detail has been overlooked cannot be over-estimated. The student is urged on no account to neglect to sketch his preparations; although it may be a little troublesome at first, it will gradually become easier.

The simplest method of sketching is that of observing the object under the microscope and then reproducing it upon paper. In doing this care should be taken to sketch in pencil and on a sufficiently large scale; the largest grains of potato starch, for instance, should measure not less than an inch in length. Sketch first the outline, and make sure that it correctly represents the outline of the grain under examination. Next put in the hilum, taking care that this also is accurately done. Then count the number of darker striations, and sketch them in their correct relative positions. Lastly, count, if possible, the number of fainter striations between two dark striations, and introduce these. Two or three grains correctly sketched form a valuable record, but a dozen grains carelessly drawn are worse than useless. Typical grains should be selected, and the number not unnecessarily multiplied by repeated sketches of very similar grains. Focus as frequently as may be necessary in order to show all the details as sharply defined as possible.

It is exceedingly desirable that the grains should be reproduced in their correct relative size. This can easily be done by means of the ocular micrometer. Suppose, for instance, the potato starch is to be sketched under a magnification of 200 diameters. Measure the grain with the ocular micrometer. It measures, say, 100μ ($= \frac{1}{16}$ mm.). It must be drawn, therefore, $200 \times \frac{1}{16}$ mm. ($= 20$ mm.) long. Mark two points on the sketching paper this distance apart. Next, measure the distance of the hilum from the nearer margin and mark its position. Lastly, measure the breadth of the grain at its widest point, and mark this. The details can then be readily filled in.

The second grain sketched can be treated in the same way. The exact magnification is known and the relative size is correctly preserved.

More commonly one of the various forms of camera lucida is used. Information concerning these can be obtained from one of the numerous works that deal specially with the microscope and its appliances, or even from the price lists of manufacturing opticians. They are not necessary for our purpose, and can therefore be dispensed with here.

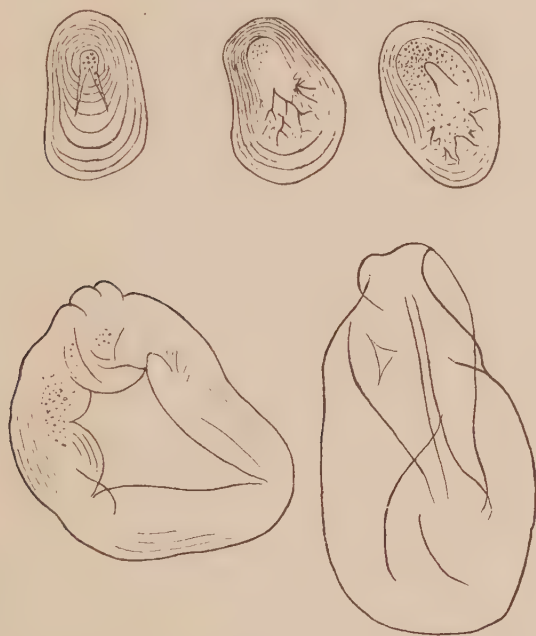


FIG. 2.—Potato Starch, showing various stages in gelatinisation.

Effect of Heat.—Mount a little potato starch in water, cover with a coverslip, and then gently warm the slide by holding it over a very small gas or spirit flame, taking care to apply the heat just beyond the coverslip. Directly the opaque starch grains near the edge of the coverslip become suddenly translucent withdraw the heat and cool the slide. Examine under the microscope, observing first the grains furthest removed from the heated end of the slide. These, or at least some of them, should be intact and show no difference from

grains that have not been so treated. Proceed towards the heated end of the slide and observe, in successive degrees, the effect of moist heat upon the grains. First the hilum fissures, and here disorganisation commences, extending, in grains that have been more affected, along the long axis of the grain, often forming a V-shaped fissure. The central portion becomes first granular, then translucent, and finally the whole grain swells considerably and is converted into a gelatinous mass in which only delicate dark lines are visible (fig. 2).

All starches gelatinise when heated with water, but the temperature at which gelatinisation is effected varies considerably with the variety of starch. The following table shows the temperatures that have been determined for some of the more important starches :

	Distinct swelling	Begins to gelatinise	Complete gelat.
Rye	45·0	50·0	55·0
Rice	53·7	58·7	61·2
Barley	37·5	57·5	62·5
Potato	46·2	58·7	62·5
Maize	50·0	55·0	62·5
Wheat	50·0	65·0	67·5
Maranta	66·2	66·2	70·0
Acorn	57·5	77·5	87·5

The temperature at which gelatinisation takes place might therefore well be used to distinguish, for instance, between rye starch and wheat starch, and the attempt has even been made to base a quantitative separation upon this property.¹

It is very important that the student should make himself acquainted with the effect of heat, and especially moist heat, upon starch grains. Many drugs, more particularly powdered drugs, are subjected to excessive heat during the process of preparing or drying, by which considerable alteration is effected in the appearance of the starch grains. These fissure or become more or less translucent in the centre, or are even entirely gelatinised.

Effect of Caustic Alkali. —Mount a little starch in water ; place a drop of solution of caustic potash on the slide near the coverslip, and gently bring it into contact with the water in which the starch is mounted. If necessary draw the alkali

¹ Weinwurm, *Ztschr. f. Untersuchung d. Nahrungs- und Genussmittel*, 1898, p. 98.

underneath the coverslip by applying a small fragment of filter paper to the opposite side. Observe the effect of the caustic alkali upon the grains. At first the striations become a little more distinct, then fainter; the central portion becomes transparent, as though solution were taking place, and finally the grain rapidly swells until the shape becomes unrecognisable.

Solution of caustic potash is by no means the only reagent that will gelatinise starch. Concentrated solutions of chloral hydrate (five parts in two of water), of calcium chloride, of zinc chloride, &c., produce a similar effect. Strong hydrochloric acid and strong sulphuric acid dissolve it.

The presence of starch in a section often obscures important details and its removal becomes desirable. Solution of potash or of chloral hydrate, or strong hydrochloric acid, will effect this, and such solutions are termed 'clearing agents.' Clearing may also be effected without the use of either acid or alkali by warming until the starch is gelatinised and then digesting with diastase until solution is effected.

Iodine Test.—Mount a fresh slide in water and irrigate with iodine water (or solution of iodopotassium iodide diluted with water to the colour of sherry). Observe that the grains assume a pale violet-blue colour, which increases in intensity till it becomes almost black.

This is the most important chemical test for starch, and should be applied in all cases in which the identity of the particles under examination appears doubtful. For its successful application the presence of water is necessary, and it should be noted that under certain circumstances the blue colour may be overlooked. This is especially the case with very minute starch grains; these are best tested by a saturated solution of iodine in the solution of chloral hydrate previously mentioned. The chloral hydrate induces gelatinisation of the grain, while the iodine colours it blue, the blue compound thus produced being insoluble in the reagent used.

Only in a few exceptional instances do the starch grains contained in a plant fail to give the characteristic blue reaction with iodine; in these cases the colour produced is reddish or violet. To such grains the name of amyloextrin (see later) has been given. They are believed to consist of amylose together with varying quantities of amyloextrin and of a dextrin that is not coloured by iodine.

Polarisation.—Mount a little potato starch in water and examine it by polarised light. For the necessary appliances and the mode of using them reference should be made to one of the text-books of the microscope. With crossed prisms each starch grain shows a dark cross upon a light ground, the point of intersection of the arms of the cross being coincident with the hilum. This behaviour of starch is to be referred to its microcrystalline structure, and is occasionally of use in detecting starch grains that might otherwise be overlooked.



FIG. 3.—Potato Starch, in polarised light. (Behrens.)

It must be observed, however, that other vegetable substances (the walls of the cells, inulin, &c.) also rotate the ray of polarised light and may exhibit a similar dark cross upon a bright ground.

Examination in Glycerin, &c.—Mount a little starch in pure glycerin; observe that the grains appear brighter and the striæ become almost invisible, while the hilum is often conspicuous as a dark spot owing to a little air being imprisoned in the hollow centre.

Repeat the experiment with oil of cloves ; the details are still less visible.

These experiments serve to show that glycerin and oil of cloves are unsuitable media in which to examine starch grains. The explanation is to be found in the fact that these liquids refract light more strongly than water does and nearly as powerfully as the starch grain itself. Were the refractive power of the mounting medium the same as that of the grain, the latter would be invisible. The same applies to cell walls and fragments of cells ; these should be examined in water for the study of minute details, as the latter become almost invisible in strongly refractive media, just as the striations of starch grains disappear in oil of cloves.

Important Starches and their Characters

(1) **Potato Starch.**—Potato starch (fig. 4) is obtained from the tubers of *Solanum tuberosum*, Linn. It is composed of



FIG. 4.—Potato Starch. $\times 240$. (Greenish and Collin.)

grains of variable size, some being so large as to be visible to the naked eye. Typical grains of this starch are flattened, and have an oval, ovate, ellipsoidal, or conchoidal outline. The hilum is punctiform and eccentric, being generally situated near the narrow end of the grain ; it is surrounded by numerous distinct concentric striations, some few of which are much more conspicuous than the others. In addition to these typical grains

there are a few others, smaller in size and rounded in outline, or rounded on one side and flattened on the other, the last named being sometimes attached by their flat sides in twos or threes.

The largest grains vary in length from $75\ \mu$ to $110\ \mu$, those of medium size from $45\ \mu$ to $65\ \mu$, and the smaller ones from $15\ \mu$ to $25\ \mu$.

(2) **Maranta Starch.**—Maranta starch (fig. 5) is obtained from the rhizomes of *Maranta arundinacea*, Linn., and other species of *Maranta*. It is commonly known in commerce as 'arrowroot'—a term, however, which is also applied to the starches of other and widely different plants.

The different varieties of arrowroot are distinguished in trade by their geographical sources. Maranta starch is known

as Bermuda, St. Vincent, West Indian, or Natal arrowroot, according to the country in which it is prepared.



FIG. 5.—Maranta Starch. $\times 240$.
(Greenish and Collin.)

The grains of Maranta starch are simple and rather large. They are irregular in shape, the smallest being nearly spherical, while the larger are rounded, ovoid, pear-shaped, or

sometimes almost triangular. The largest bear numerous fine concentric striations, and a conspicuous rounded, linear or stellate, eccentric hilum. In some varieties of arrowroot (Natal) the rounded hilum predominates, in others (St. Vincent) the linear or stellate, the latter often resembling the wings of a poised bird. The grains average about $30\ \mu$ to $40\ \mu$ in length, but may attain to $45\ \mu$, $60\ \mu$, or even $75\ \mu$, as, for instance, in Bermuda arrowroot; the smaller grains vary from $7\ \mu$ to $15\ \mu$.

(3) **Maize Starch.**—The starch obtained from the fruits of *Zea Mays*, Linn.

Take a grain of maize and cut it longitudinally into two parts. The centre of the grain is white and mealy; on one side of the whitish part, or partially surrounding it, is a yellowish

horny portion, while on the other side is a greyish portion in which the embryo and scutellum can be discerned; the whitish mealy and yellowish horny portions constitute the endosperm. The reserve starch with which the endosperm is filled forms the maize starch of commerce.

Remove a little of the starchy central portion and examine it in water.

The starch grains appear rounded or muller-shaped; a few are polygonal. They are simple, and vary in size from 5 to 20 μ , but are on the whole tolerably uniform, the majority measuring from 12 to 18 μ . The hilum is mostly distinct, sometimes as a point, but more often as a two-, three-, or four-armed cleft; striations are not discernible.

Cut off from another grain a little of the horny portion of the endosperm and soak it in water for twenty-four to forty-eight hours. It will soften. Remove a small portion about the size of a pin's head and break it up with the dissecting needles in a drop of water. Examine it.

The starch grains are partly free, partly still contained, closely packed, in the cells of the endosperm.

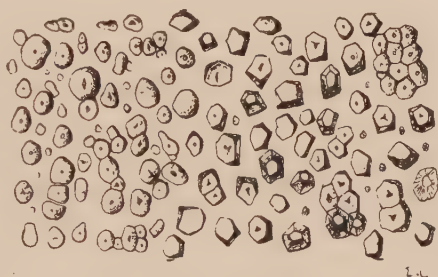


FIG. 6.—Maize Starch. $\times 240$.
(Greenish and Collin.)

The free grains are, like those in the previous preparation, simple and tolerably uniform in size, but they differ in being mostly polygonal with rather rounded angles. In outline they are often pentagonal. The hilum is frequently a point, and often exhibits two, three, or four radiating clefts. Some grains are much fissured at the periphery. In others there appears to be a large central cavity or possibly gelatinous portion surrounded by a brighter peripheral layer, the latter being often radially striated or fissured. These grains are usually larger than the others, and appear to have undergone partial gelatinisation. In none of the grains can concentric striation be detected.

All these grains may be found in commercial maize starch, but those with large central cavity are few in number.

(4) **Rice Starch.**—Rice starch is obtained from the fruits of *Oryza sativa*, Linn.

Soak a few grains of rice in water for three or four hours, then scrape off a little of the softened grain and mount it in water.

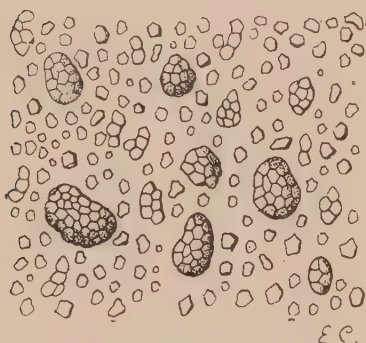


FIG. 7.—Rice Starch. $\times 240$.
(Greenish and Collin.)

The starch grains are very small and angular; their sides are mostly flat, but occasionally they are curved. There is seldom any hilum distinctly visible, but in some grains a central portion appears brighter—a difference possibly due to the drying of the grain. They are regular in appearance and uniform in size, averaging about 6μ in diameter, but grains up to 8μ or even 10μ may be found,

as well as some that are very minute.

Among these angular grains there will be found larger masses consisting of a number of grains compacted together (compound grains); they are ovoid or nearly spherical in shape, and measure from 20 to 30μ in length. By pressure

they easily break up into their component grains, and hence they are seldom found intact in commercial rice starch.

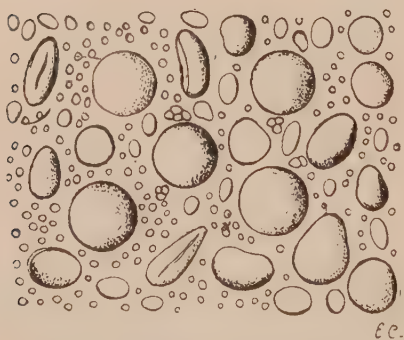


FIG. 8.—Wheat Starch. $\times 240$.
(Greenish and Collin.)

(5) **Wheat Starch.**—Wheat starch is obtained from the fruits of several species of *Triticum*.

Soak a few grains of wheat in water for twenty-four hours. Cut one transversely, and mount a little of the starch in water.

Examine it with the low power; observe that it consists of large rounded grains mixed with numerous small ones; grains of intermediate size are comparatively rare.

Examine with a high power. The large grains appear

rounded or nearly oval without evident hilum or striations, but on careful examination one may be found here and there with a distinct hilum in the shape of a point, or cleft, or apparent cavity, and an occasional grain will also exhibit delicate concentric striation. In some samples of wheat most of the grains may show distinct striation.

Make the grains roll by moving the coverslip or by pressing on one side of it. As the large grains roll they will appear oval or concavo-convex in outline, showing that the grains are not spherical but lenticular or bun-shaped; some will exhibit a darker longitudinal line in the centre, corresponding to the central cavity previously mentioned.

In diameter the large grains measure mostly from 20 to 35 μ (when lying flat).

(6) **Rye Starch.**—Rye starch may be obtained from the fruits of *Secale cereale*, Linn. Rye starch closely resembles wheat, but the large grains attain a larger size (45 to 50 μ), and many of them exhibit a dark central cavity with several radiating clefts.

(7) **Barley Starch.**—Barley starch is contained in the fruits of *Hordeum distichon*, Linn. Barley starch also closely resembles wheat, but the large grains are rather smaller, the majority measuring 18 to 25 μ , a few as much as 30 μ . They are also less

regularly circular, showing a tendency to bulge on one side and thus assume a sub-reuniform shape (compare pea starch). In side view they are elliptical or lemon-shaped rather than lenticular.

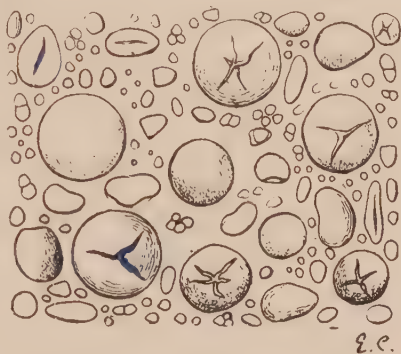


FIG. 9.—Rye Starch. $\times 240$.
(Greenish and Collin.)

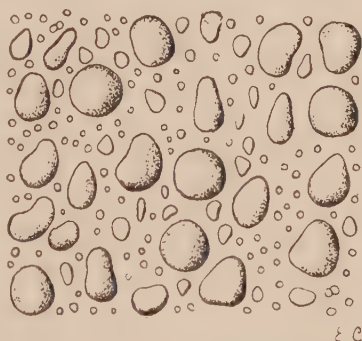


FIG. 10.—Barley Starch. $\times 240$.
(Greenish and Collin.)

Note.—Wheat starch is a common article of commerce, but rye and barley starches are not. The detection of barley starch when mixed with wheat starch is difficult, but the identification of the corresponding flours is easier, for these always contain fragments of the pericarp, &c., which offer additional and very valuable evidence of identity (compare 'Wheat,' in Section X).

(8) **Oat Starch.**—Oat starch is contained in the fruits of *Avena sativa*, Linn. It is not met with as an article of commerce, but an examination of the starch is desirable, as oatmeal is used for a variety of purposes.

When examined under the low power it appears to consist of a mixture of large and small grains, but when the former are

examined under the high power they are seen to be compound grains consisting of a large number of small angular grains, into which they readily separate. In this respect the starch resembles rice starch.



FIG. 11.—Oat Starch. $\times 240$
(Greenish and Collin.)

Most of the component grains are angular, but some are lemon-shaped, rounded or semi-circular; these form a valuable means of distin-

guishing the starch from rice starch. Neither hilum nor striæ are visible.

The simple grains average about $10\ \mu$ in diameter and the compound 35 to $45\ \mu$.

Oatmeal contains fragments of the seed-coats, pericarp, and paleæ, which may be utilised to establish the identity of the meal.

(9) **Bean Starch.**—Prepare a little starch from a haricot bean (*Phaseolus vulgaris*, Linn.) in the same manner that maize and rice starch were prepared.

The larger grains are ovoid or elliptical in outline or somewhat reniform; sometimes they are obscurely three- or four-sided, with very rounded angles, the smaller grains being often

rounded. Through the hilum there usually extends along the long axis of the grain almost from end to end a large, irregular, branching cleft that appears nearly black and is therefore very conspicuous; more delicate fissures often radiate towards the periphery. The striae are usually well marked. In length the grains vary from 25 to 60 μ , 30 to 35 μ being common measurements.

Examined in glycerin or in alcohol the grains exhibit no cleft, but this gradually appears when they are irrigated with water. This phenomenon is probably due to swelling of the grain in contact with water.

The starches of the commoner leguminous food stuffs (pea, bean, lentil, &c.) exhibit a remarkable similarity in size and shape. It is not, therefore, very difficult to recognise such a starch as being derived from a leguminous plant, but the determination of the species that has yielded it is by no means easy (compare the descriptions and illustrations of leguminous flours in Section IX.).

(10) **Pea Starch.**—The starch of the seeds of *Pisum sativum*, Linn. The grains of pea starch are rather smaller than those of bean starch, the majority measuring from 20 to 40 μ . Some of the grains are oval-oblong, others rounded or sub-reniform in shape; many are irregularly enlarged, so that their outline appears composed of arcs of circles of varying radius. The appearance of the hilum varies. In some specimens there is a conspicuous dark central cleft, as in bean starch; usually, however, it is less branched, smaller, and less conspicuous. In others there are but few grains with such clefts, the majority possessing simply an elongated hilum. Dark transverse clefts are also occasionally to be found. Radial fissures are comparatively common. Most grains are also distinctly striated, especially near the periphery, the central portion often presenting a more or less homogeneous appearance.

(11) **Lentil Starch.**—The starch of *Lens esculenta*, Moench.

Lentil starch is intermediate in character between pea starch and bean starch. Most of the grains are simple and elliptical, ovoid, or rounded in shape; some of them exhibit irregular enlargements, like those of the typical grain of pea starch. The hilum is less branched and less conspicuous than that of bean starch, and usually not so dark; it often appears as a dark cleft extending almost the entire length of an oval grain, which then

bears some resemblance to the flat side of a coffee bean. The striations are delicate, but they are usually visible. Most of the grains measure from 20 to 40 μ in length.



FIG. 12.—Tous-les-mois Starch. $\times 240$.
(Greenish and Collin.)

(12) **Tous-les-mois.**—The starch known as Tous-les-mois or Queensland arrowroot is obtained from the rhizomes of *Canna edulis*, Linn., and other species of *Canna*. The grains recall those of potato starch, but they are much larger, the average length being 60 to 95 μ , although they occasionally reach 130 μ .

In shape they are broadly ovoid, oblong, or elliptical; many are flattened at the anterior extremity, and bear

in addition a slight prominence opposite the hilum. In some grains similar prominences are observable on the sides, in others there is a distinct shallow depression on one side. The hilum is usually a point, often dark and in most grains very eccentric



FIG. 13.—Curcuma Starch. $\times 240$.
(Greenish and Collin.)

($\frac{1}{8}$ to $\frac{1}{4}$). The striæ are very distinct and regular. The grains differ, therefore, from potato starch in size, in shape, and in striation.

(13) **Curcuma Starch.**—The starch obtained from *Curcuma angustifolia*, Roxb., and other species of *Curcuma*. It is often termed East Indian arrowroot. The grains are broadly ovoid or oblong, sometimes

inclining to reniform or sub-reniform; they are usually rounded at the posterior extremity, but often taper rather abruptly at

the other, frequently terminating in a nipple-like projection, in or near which the hilum is situated; the latter is commonly very eccentric; when not visible its position can be ascertained by following the concentric striae, which are usually distinct, at least in the large grains. The grains are so flat that when viewed on their edges they appear rod-shaped. The average size is from 36 to 60 μ .

Many Scitamineaceous plants contain a starch of this type.

(14) **Ginger Starch.**—The starch of *Zingiber officinale*, Roscoe. Ginger starch resembles curcuma starch in general aspect. The grains are, however, smaller, varying mostly from 20 to 35 μ in length; they are rather thicker, and bear a distinct resemblance to a sack tied at the neck.

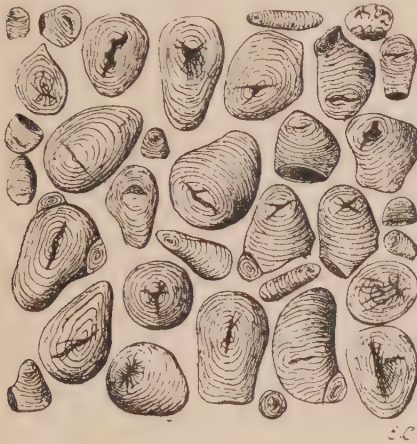


FIG. 14.—Sago Starch. $\times 240$.
(Greenish and Collin.)

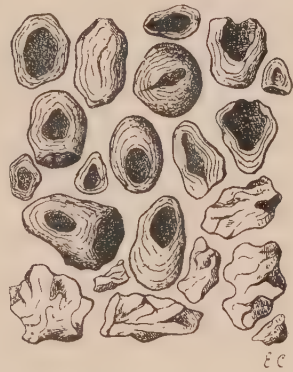


FIG. 15.—Sago. $\times 240$.
(Greenish and Collin.)

(15) **Sago.**—Sago is prepared from the moist starch of the sago palm, *Metroxylon Sagu*, Rottb., by heating and stirring it until it agglomerates into small rounded granules; this form of the starch is often distinguished as pearl sago.

Soak a little sago in water for a few hours; crush a granule or two on the slide with water, and examine.

Some of the starch grains have been more or less gelatinised by moisture and heat.

Observe, first, the starch grains that are intact or nearly so. They are ovoid, broadly ovoid, muller-shaped, or irregularly three- or four-sided with rounded angles. Some are simple, but others bear, on as many short protuberances, one, two, or three flattened

surfaces to which smaller grains have been attached, thus forming compound grains. Small grains may occasionally be found presenting one flat surface. The hilum is eccentric, but is usually more or less altered by gelatinisation. In most of the grains the striations are only indicated, but in some they are very distinct. In length the intact grains vary from 30 to 50 or 60 μ .

Next proceed to study the grains that have been altered to varying degrees by the moist heat to which they have been subjected. In many of these gelatinisation has commenced near the hilum, and converted the central portion into a transparent gelatinous mass; in such grains fissures may be seen leading towards the posterior extremity. In others a considerable proportion (one-fourth or one-third) of the grain has been gelatinised; others have been converted into a gelatinous mass in



FIG. 16.—Tapioca Starch. $\times 240$.
(Greenish and Collin.)



FIG. 17.—Tapioca. $\times 240$.
(Greenish and Collin.)

which but little structure is visible, although the original shape may be to some extent retained; these have been usually enlarged by swelling. Lastly, numerous *débris* of completely gelatinised grains may be found (compare the experiment on the gelatinisation of potato starch).

(16) **Tapioca.** —Tapioca is prepared from the starch obtained from the tubers of *Manihot utilisima*, Pohl. The method of preparation resembles that of sago.

Treat tapioca as directed for sago.

Examine the intact grains first; they are small, and the presence of one or two flat surfaces points to their having formed component parts of compound grains. Many are muller-shaped; they often have one flat surface, sometimes two, forming an angle where they meet. The hilum is usually a point or small cleft, and eccentric. The grains that appear muller-shaped when lying on their sides are circular when stand-

ing on end, and in the latter case the hilum appears central. Striations are visible only in some of the larger grains.

The majority of the ungelatinised grains vary from 15 to 25 μ in length, but they may reach as much as 36 μ , those that are gelatinised being much larger.

Many of the grains exhibit the effects of heat similar to those shown by sago. The centre first becomes gelatinous, then radial fissures arise, especially in the peripheral portion. In many grains that are completely swollen the original shape of the grain is distinguishable; others have become converted into a shapeless gelatinous mass.



FIG. 18.—Amylodextrin of Mace. (Tschirch.)

Dextrin.—Dextrin can be conveniently examined in alcohol, in which it is insoluble and which effects no appreciable alteration in it. The starch grains from which it has been prepared have usually suffered but little visible change, and the variety can therefore be easily identified; usually it is potato starch.

On irrigating with water the grains swell a little and become translucent, the change progressing from the periphery towards the centre; the striations become very conspicuous, the grains partially dissolve, and finally solution becomes almost complete.

Amylodextrin.—Here and there in the vegetable kingdom, among drugs and spices, particularly in mace, parenchymatous cells are to be observed which contain small irregular granules. These granules vary exceedingly in shape. They are often

elongated, with irregular wavy outline, and not unfrequently exhibit rounded or oval protuberances. They are seldom oval or rounded, as starch grains are. In size they range from 5 to 15 μ . They are characterised also by yielding a reddish-brown or reddish-violet colouration with iodine, a reaction which distinguishes them at once from starch grains.

These remarkable granules are believed to consist of amylose together with amylopectin and a variety of dextrin that is not coloured by iodine. Amylopectin is intermediate between starch and dextrin.

Notes on the Examination of Starch

The adulteration of one starch with another is detected by mounting a carefully bulked sample in water, examining with the microscope, and comparing, if necessary, the grains observed with a sample of the same starch of known genuineness. No report should be given without, if possible, such comparison.

The detection of starch as an adulterant of other (especially powdered) drugs should invariably be confirmed by the iodine test, in order to avoid mistaking minute particles of other substances (sand, &c.) for starch grains.

In the examination of a starch for substances other than starch it is often advisable to remove by suitable means the starch that is present, and thus concentrate into a small compass any foreign substance present. This may be effected by either of the following methods :

- (a) Mix 5 grammes of the starch with 10 grammes of 25 per cent. hydrochloric acid, warm to 40° C., dilute with 10 grammes of water, and allow the insoluble particles to subside ;
- (b) Boil 2 grammes of the starch with 20 c.c. water, cool to about 40° C., and add 10 c.c. of a filtered infusion of malt ; keep the mixture at about 40° C. until the gelatinised starch is dissolved. It can then be set aside to deposit.

In both cases the use of a centrifuge will rapidly effect the separation of the deposit, which can thus be quickly washed and examined.

Compare also the isolation of the fragments of bran from wheat flour (Section X.).

SECTION II

HAIRS AND TEXTILE FIBRES

INTRODUCTION

THE substances that were the subject of investigation in Section I. required but very little preparation to bring them into a fit state for microscopical examination, and their structure was so simple that this examination was very easy. With textile fibres the case is rather different. Not many of them are met with in such a condition that they can be mounted and examined without previous preparation, and often both careful and accurate observation is required before the minute differences that distinguish one fibre from another can be detected. Even to the experienced microscopist the identification of a textile fibre frequently presents considerable difficulty.

Cotton and flax fibres frequently find their way into microscopical preparations, and it is therefore desirable that the student should be able to recognise them. Hemp, jute, and Manila hemp are among the most valuable textile fibres. Wool is an example of a common animal fibre. It is not intended that the student should do more now than make himself familiar with the general characters of the most important fibres and the methods adopted in examining them. Those that desire to pursue the study of this important subject further must be referred to works¹ dealing specially with it.

Cotton Wool

Source.—Cotton wool consists of the hairs that cover the seeds of various species of the *Gossypium*. These hairs are

¹ Wiesner, *Rohstoffe des Pflanzenreiches*, Vienna, 1902, Parts 7 and 8; von Höhnelt, *Die Mikroskopie der technisch-verwendeten Faserstoffe*, 1887.

separated from the seeds by machinery, and, after passing through various processes, are spun into yarn.

Mounting—Procure, if possible, a little raw cotton, or, failing that, a little ordinary non-absorbent cotton wool. Moisten a few threads with a drop of alcohol on the slide, allow most of the alcohol to evaporate, add a drop of water, cover with a coverslip, and examine under the higher power.



FIG. 19.—Cotton, magnified. (Tschirch.)

Examination—The hairs resemble more or less twisted ribbons, the edges of which are considerably thickened (figs. 19, 20, *a*, *b*). The surface is not quite smooth, but appears delicately granular or striated, the striations being frequently oblique; these markings are on the cuticle which covers the hairs. With a little searching an apex as well as base of a hair may be found and examined.

Cotton hairs attain a very considerable length, ranging from 10·3 mm. (Bengal cotton) to 40·5 mm. (sea island cotton).

In transverse section they exhibit a flattened, reniform, or irregular outline, and a narrow elongated cavity; they vary from 0.011 to 0.042 mm. in width (Wiesner), and differ from many other fibres in being always isolated and never exhibiting a circular or polygonal section.

The student should sketch the middle portion and apex of a hair.



FIG. 20.—Cotton Fibres. *a, a, a*, central portions of mature hairs; *b*, weaker hair; *c*, strongly thickened hair; *d*, apices of hairs; *e*, dead hair. (Hanausek.)

Reactions.—Irrigate a few hairs mounted in water with freshly prepared cuoxam (see list of reagents); they rapidly swell and dissolve, with the exception of a very thin, delicate membrane, the cuticle, which is insoluble. If the reagent is not too concentrated, and the swelling not too rapid and vigorous, the cuticle will encircle the swollen hair at intervals and produce constrictions sharply alternating with large expansions.

Mount a fresh preparation in water, irrigate with solution of iodine in potassium iodide (a 1 per cent. aqueous solution of potassium iodide, saturated with iodine, v. Höhnelt, 1887);

allow the reagent sufficient time to be absorbed by the cotton ; remove the excess by filter paper so that the cotton is nearly dry, then irrigate with sulphuric acid (concentrated sulphuric acid 3 volumes, water 1 volume, glycerin 2 volumes, v. Höhnel). The hairs assume a blue colour (cellulose reaction), whilst the dried protoplasm in the cavity remains brown.

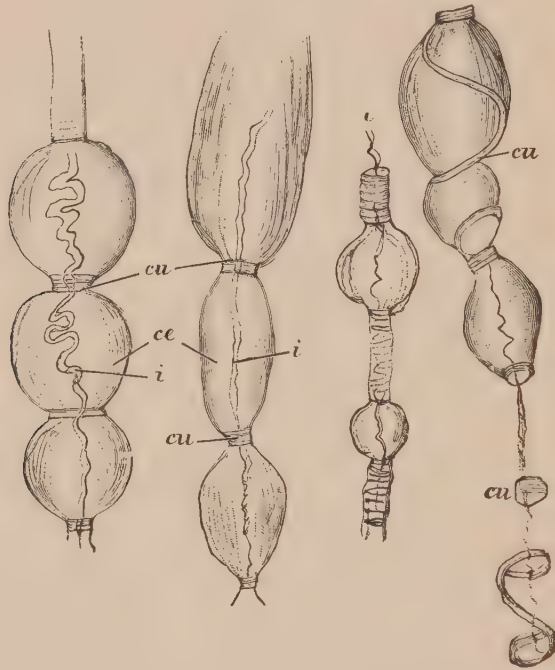


FIG. 21.—Cotton Fibres in Cuoxam. *ce*, cellulose wall swollen by the reagent ; *cu*, encircling rings of cuticle which are not swollen ; *i*, inner membrane lining the cavity. (Hanausek.)

Mount a fresh preparation in water, and irrigate with solution of chlorzinciodine ; the hairs gradually acquire a bluish-violet colouration (cellulose reaction).

Mount a few threads in a saturated aqueous solution of picric acid, warm gently for a few moments, and cool ; the hairs are not stained yellow (distinction from animal wool, which stains yellow in hot solution of picric acid).

Flax

Source.—Flax consists of the bast fibres from the stem of *Linum usitatissimum*, Linn. These are separated from the plant by the processes of retting, scutching, heckling, &c., for a description of which reference should be made to one of the works dealing specially with the subject.

Mounting.—Procure from a rope-maker a little flax ; cut off a few fragments, and examine them in water or chloral hydrate. The threads consist chiefly of *groups* of bast fibres. Here and there fragments of cell *débris* can be seen adhering to the fibres ; these are the remains of the parenchyma of the flax stem, which has been imperfectly removed from the fibres. Particles also of cell contents of a greenish colour often remain attached to the fibres, and obscure their minute details.

Separation of the Fibres.—Boil a little flax in a 10 per cent. solution of sodium carbonate for half an hour, using a reflux condenser to prevent the solution from becoming too concentrated ; pour off the alkaline solution, wash the flax with distilled water, and preserve it in dilute alcohol.

Take a little, and separate the constituent bast fibres by pulling them apart with the fingers, or by carefully teasing them out with the needles in a drop of water on the slide. Remove the water, mount in a drop of dilute glycerin, and examine.

Examination.—The individual fibres are now more or less completely

separated from one another and freed from the cell *débris* and contents that were previously adhering to them. Under a high power the walls of each fibre are seen to be very thick,

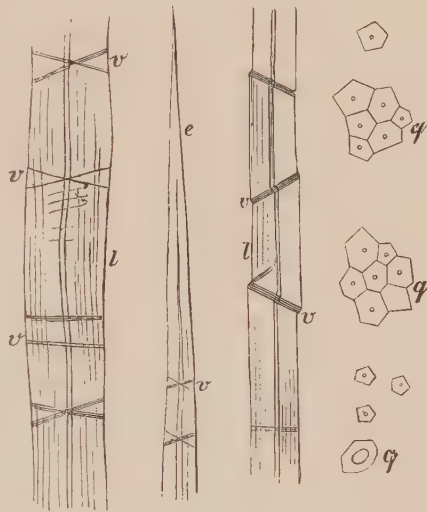


FIG. 22.—Flax Fibres. *l*, longitudinal aspect, $\times 400$; *q*, transverse section, $\times 200$; *e*, apex of a fibre; *v*, transverse markings. (von Höhnelt.)

and faintly but distinctly striated; the cavity is narrow, uniform in width, and contains a little granular matter (remains of protoplasm).

Examine the walls minutely. Here and there delicate oblique lines can be detected, often crossing one another (fig. 22, *v*), and frequently the fibres show slight local enlargement; these appearances are due to injuries inflicted by the mechanical processes to which the fibres have been subjected, and may therefore vary in frequency in different varieties of flax. The uninjured bast fibres of the plant exhibit neither longitudinal nor transverse striation nor protuberant swellings.

Mount a few prepared threads in chloral hydrate; the transverse lines are more distinct. Examine this preparation with the low power, and search for the tapering pointed ends of the fibres; a few of these are generally to be found. Sketch a portion from the middle of a fibre, and also one of the ends.

Reactions.—The treatment with sodium carbonate does not appreciably interfere with the reactions of the fibre, of which the following are the most important:

Stain a little of the prepared and disintegrated fibres with iodine and sulphuric acid, as detailed for cotton. The fibres slowly assume a fine blue colour; the transverse lines, being the first part to colour, are at that moment particularly conspicuous. The cavity contains a yellowish-brown granular substance (remains of protoplasm).

Mount a little of the flax in water and irrigate with cuoxam; the fibres swell and dissolve at once, leaving a minute thread from the centre undissolved; in dissolving they do not exhibit the sharp constrictions characteristic of cotton hairs, since the fibres are not covered by a cuticle.

Preparation of Transverse Sections.¹—Prepare transverse sections of some flax fibres as follows:

Take a number of threads of flax, without previous treatment with sodium carbonate, and draw them between the fingers until they are fairly straight; then moisten them with gum

¹ For the sake of completeness the directions for preparing transverse sections are introduced here, but the student who has not had considerable experience in section-cutting is recommended to content himself for the present with the separation and examination of the fibre.

Directions for cutting sections will be found under 'Ergot.'

and glycerin and make them into a single rounded strand about as thick as a blanket-pin, taking care to keep the fibres straight. Let this strand dry thoroughly (in a warm place for twenty-four hours) : fix it firmly in cork, and cut with a sharp razor transverse sections as thin as possible. Transfer these to a slide ; drop on alcohol to free them from air, and mount in glycerin or a mixture of glycerin and alcohol ; water may also be used, but many of the fibres, released from adhesion to the others, will assume a horizontal or oblique position.

The section will exhibit a number of groups of bast fibres, each group consisting of several fibres. The fibres are very thick-walled, uniformly polygonal in outline, and have very small cavities. There are no intercellular spaces.

Diagnostic Characters.—The following are the chief diagnostic characters of flax fibres :

- (a) The transverse sections are uniformly polygonal, and have very small rounded cavities ; the fibres are usually grouped ;
- (b) The fibre consists entirely, or almost entirely, of cellulose ; hence it colours pure blue with iodine and sulphuric acid and dissolves in cuoxam ; there is no cuticle ;
- (c) The fibres exhibit transverse lines ;
- (d) The ends taper gradually to fine points.

Hemp

Source.—Hemp is obtained from *Cannabis sativa*, Linn. The fibres are separated from the stem by a method similar to that adopted for flax.

Preparation and Examination.—Prepare hemp for examination as directed for flax.

The fibres can also be separated by macerating in a 10 per cent. solution of chromic acid in dilute sulphuric acid until a strand taken out readily separates into its constituents ; the remainder can then be washed, and examined as directed for flax.

Diagnostic Characters.—Hemp fibres very closely resemble flax ; they show similar swellings and transverse lines, and

also consist almost entirely of cellulose. They may be distinguished by the following features :

- (a) In transverse sections the cavities are comparatively large and oval or elongated ;
- (b) The ends of the fibres are usually blunt or almost spatulate, and sometimes forked ;
- (c) The coarser varieties often have cell *débris* attached to them, among which cells with brown contents, portions of the epidermis with warty hairs, and cells with rosettes of calcium oxalate are to be noted as distinctive.

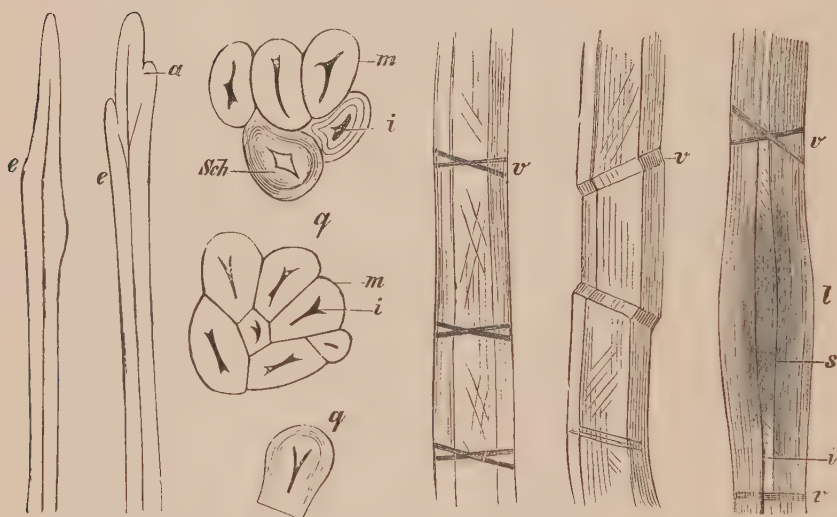


FIG. 23.—Hemp Fibres. On the left the apices of two fibres ; on the right, portions from the middle ; in the centre, transverse sections. *a*, short branch near the apex ; *i*, lumen ; *m*, middle lamella ; *s*, *Sch*, striations ; *v*, transverse or oblique markings. $\times 325$. (von Höhnelt.)

Jute

Source.—Jute is obtained from *Corchorus olitorius*, Linn., and *C. capsularis*, Linn.

Preparation and Examination.—As directed for flax.

Diagnostic Characters.—

- (a) Jute fibres are quite smooth ; they show no longitudinal striations and no transverse lines ;

- (b) They are particularly distinguished by the size of the cavity, which is not uniform throughout the length of the fibre, but exhibits at intervals contractions caused by a corresponding increase in the thickness of the walls, which may occasionally be so great as to completely obliterate the cavity; this variation in the size of the cavity is conspicuous in the transverse

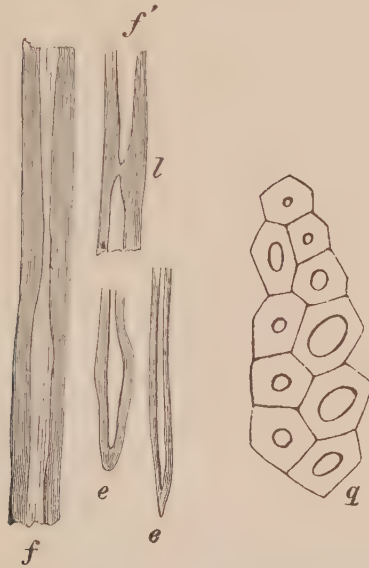


FIG. 24.—Jute. *e, e*, apices of fibres; *f*, fibre with constricted lumen; *f'*, fibre with septate cavity; *g*, transverse sections of fibres. (Hanausek.)

sections of the fibres, some of which have large and others small cavities;

- (c) They are lignified;

- (d) The ends of the fibres are blunt, sometimes almost spatulate.

Manila Hemp

Source.—Manila hemp is obtained from *Musa textilis*, Née.

Preparation and Examination.—As for flax.

Diagnostic Characters.—

- (a) The fibres are smooth, and show neither longitudinal nor transverse markings or swellings ;
- (b) The cavity is large and uniform, the fibres gradually tapering to fine pointed ends ;
- (c) The walls are lignified ;

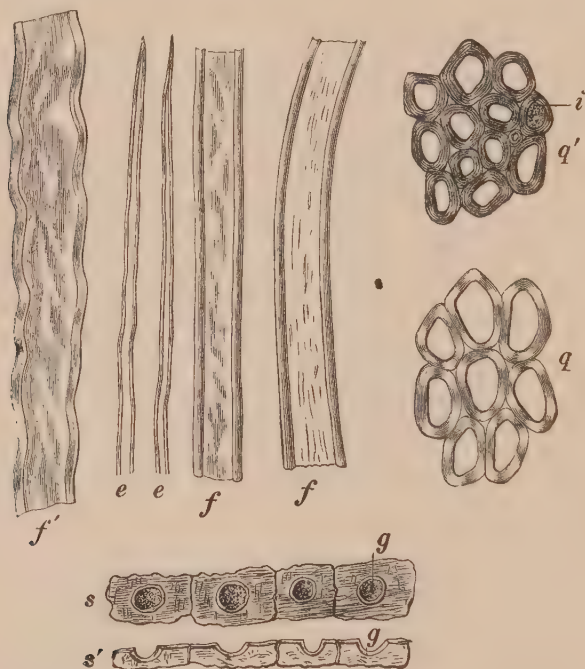


FIG. 25.—Manila Hemp. *e, e*, apices of fibres; *f, f*, central portions; *f'*, crushed fibre; *q*, transverse section of coarse fibres; *q'*, transverse section of fine fibres; *i*, fibre with proteid contents; *s, s'*, stegmata with depressions, *g*. (Hanausek.)

- (d) The fibres are often accompanied by stegmata.¹
- (e) In section they are rounded or polygonal, and show large cavities and small intercellular spaces ;

¹ Stegmata are cells that accompany the bast fibres of certain plants and remain attached to them when the fibres are separated from the stems. They occur only in ferns and monocotyledonous plants. Among the textile fibres those derived from Musaceæ, Pandanaceæ, and Palmeæ are alone characterised by the presence of stegmata. They generally contain nodules of silica, which may be found in the ash of the fibre, but the cell wall is not usually siliceous. (Wiesner.)

Sheep's Wool

Source.—The raw unwashed wool of the sheep.

Preparation.—If a few fibres of raw wool be examined in water, droplets of fatty matter will be seen adhering to them; these can be stained with tincture of alkanna (for details compare 'Lycopodium'), and consist of the wool fat naturally present in the fleece. It is desirable that this be removed before the wool is examined.



FIG. 26.—Various Forms of Fibre from Sheep's Wool. (Hanausek.)

Boil a little raw wool in water, and pour off the cloudy liquid. Wash the residual wool with a little alcohol, and finally defat it with alcohol-ether or with chloroform. Allow the defatted wool to dry.

Examination.—Mount a little in water. Each fibre exhibits an outer layer of overlapping flattened cells with wavy walls; within these cells there is a fibrous tissue, and in the centre often a pith; the latter is not always visible.

Mount a little in cuoxam, warm gently, cool, and examine;

the fibres stain bluish violet and the fibrous structure becomes very distinct ; they do not swell or dissolve.

Mount another portion in solution of picric acid and warm ; wash out with water ; the fibres are stained yellow.

Stain another portion with iodine and sulphuric acid, as directed for cotton ; the fibres stain yellow ; they do not dissolve even when gently warmed.

Mount another portion in pure concentrated sulphuric acid and warm gently ; they do not dissolve.

These reactions are amply sufficient to distinguish animal wool from the foregoing vegetable fibres. The identification of the source of the animal wool is, however, a matter of difficulty ; in many cases it is at present impossible.

SECTION III

SPORES AND GLANDS

INTRODUCTION

THE drugs that are treated of in this section do not require any elaborate preparation to fit them for microscopical observation. They differ from the substances that have already been discussed, inasmuch as their contents require particular examination.

Lycopodium

Source.—Lycopodium is a pale yellow, very mobile powder consisting of the spores of *Lycopodium clavatum*, Linn., and probably other species.

Mounting.—When thrown upon the surface of water lycopodium floats, and hence is with difficulty mounted in that medium for microscopical examination. The following method of procedure is the best: Place a small drop of alcohol on the slide, and mix a little lycopodium with it; allow most of the alcohol to evaporate, and then add a drop of dilute glycerin. The lycopodium will now mix easily with the dilute glycerin, and be free from the air bubbles which otherwise cling to it so pertinaciously.

Examination.—Examine first under the low and then under the high power. Observe the characteristic shape of the spore; it resembles a low, broad, triangular pyramid resting upon a convex base; this shape is evidently produced by the mutual pressure of the spores in the cell in which they were formed. Examine the delicate network that covers the convex base of each spore, and extends over the flat sides nearly but not quite to the angles they make with one another. This network consists of raised, colourless, transparent ridges, which

appear as reticulate markings on the surface of the spore, but are seen on the edge to project above it. From the edge of the spore teeth appear to stand out, and between these a delicate membrane is stretched; the teeth are the raised ridges seen edgewise, the membranes are the ridges seen lengthwise.

Sketching.—Proceed next to sketch three or four spores, selecting such as are lying in different positions so that the sketch may represent different aspects of the spore. Draw the outline of the spore first. Next count the number of reticulations that are visible both transversely and longitudinally; introduce these into the sketch, taking care to represent their shape as accurately as possible and preferably by single, not double, lines. In counting the number of reticulations raise and lower the tube of the microscope, if necessary, so as to focus successively each part of the surface observed. On the curved base, for instance, the reticulations are not all upon the same plane, and therefore cannot all be distinctly seen at one and the same moment. They are nevertheless represented on the sketch as though that were the case.

Examination of Contents.—Mount a fresh slide, using water in the place of dilute glycerin. Press the coverslip firmly on to the slide with the handle of a pen or of a brush, so as to crush some of the spores. Examine the slide under the microscope. Some of the spores will have burst, usually along one of the angles, and discharged their liquid contents, which assume the form of globules. If the spores do not burst, repeat the pressure on the coverslip.

Identify these globules as fixed oil by the following characters and reactions:

- (1) They appear as globules of *uniform* colour bounded by a *narrow* dark line; there is no broad black border or bright centre (distinction from air bubbles).
- (2) Irrigate the slide with tincture of alkanna diluted with an equal volume of water at the moment of using; the globules absorb the colouring matter and assume a reddish tint.
- (3) Irrigate a fresh slide with a drop or two of solution of osmic acid; the globules rapidly assume a dark brown colour, the whole spore gradually colouring as the reagent penetrates and comes in contact with the oil that it contains.

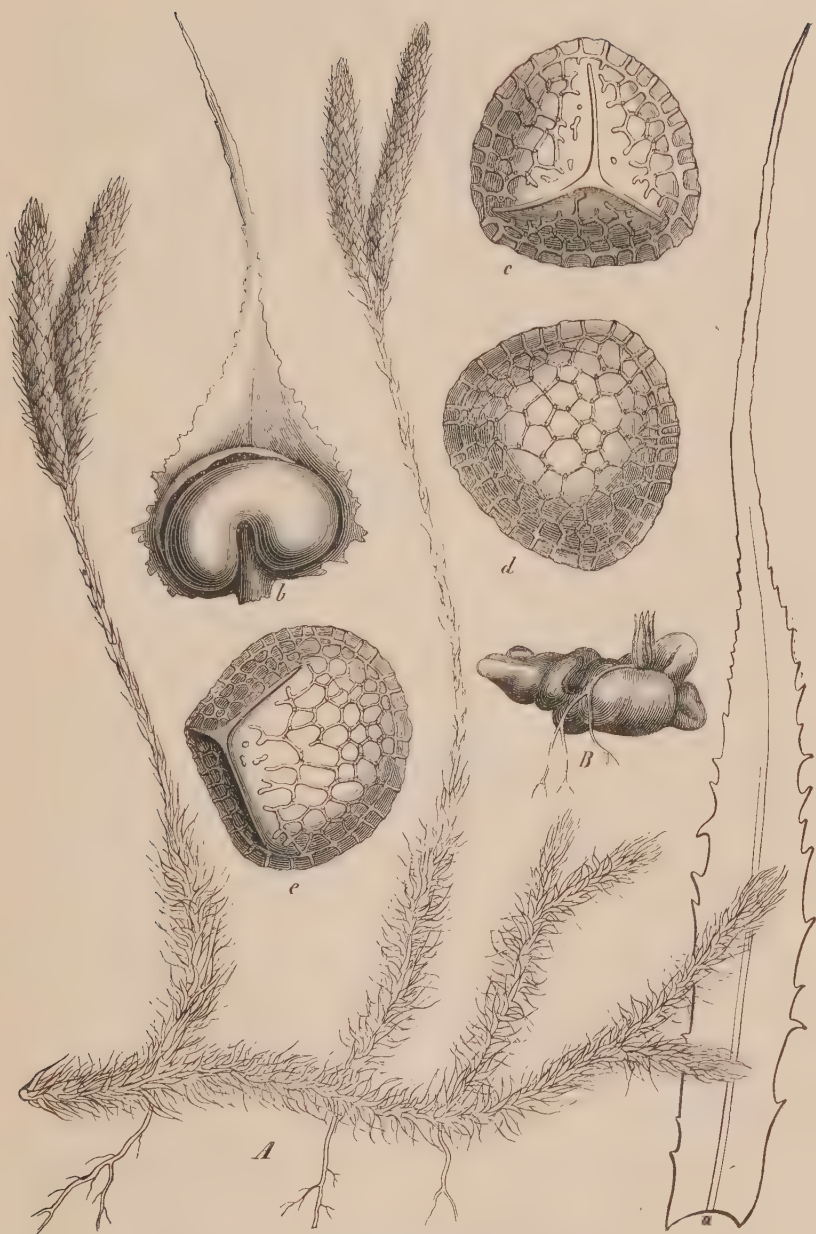


FIG. 27.—*Lycopodium*. *A*, portion of *Lycopodium clavatum*, Linn. (natural size). *a*, foliage leaf; *b*, sporophyll, with sporangium magnified *c*, *d*, *e*, spores magnified about 600 diameters; *B*, prothallium of an allied species. (Luerksen.)

- (4) Irrigate a fresh slide with solution of Soudan red ; the globules are coloured brilliant red.

The reactions 2, 3, and 4 are the principal colour reactions for fixed oil, and should be carefully studied. Volatile oils yield the same reactions, but may be distinguished by their ready solubility in alcohol.

Adulterations.—The most frequent adulterations of lycopodium are starch and the pollen of various plants, especially of coniferous trees (pine pollen).

The pollen grains of most Abietæ bear on each side a hollow vesicle produced by dilatation of the exine ; hence their shape is extremely characteristic.

The pollen grains of the hazel are nearly spherical and exhibit three pores. Those of most other plants differ so notably from lycopodium spores as to be distinguishable at first sight.

Starch may be recognised by its appearance and identified by the iodine test.

Inorganic matter can also be detected under the microscope, but in this case chemical examination is to be preferred (determination of the ash, &c.)

Lupulin

Source.—Lupulin is a yellowish-brown granular powder consisting of the glands obtained from the strobiles of the hop, *Humulus Lupulus*, Linn.

Mounting.—Procure some fresh hops ; from one of the strobiles remove some of the bracts ; these may be recognised by the minute fruit enfolded at the base. Examine a bract with a hand lens, and observe the pale or golden yellow glands that are scattered over the base ; this is lupulin.

Transfer some of the glands to a slide, using a needle or small brush for the purpose. Moisten with alcohol, and *immediately* add a drop of glycerin. Do not allow the alcohol to remain long in contact with the glands, as it rapidly penetrates them and dissolves the contents. Lower the coverslip carefully, for even slight pressure is sufficient to burst the glands and cause them to discharge their contents.

Examination.—Examine them first under the low power. Each gland consists of a single hemispherical layer of cells, the common cuticle of which has been raised, dome-like, by the

secretion of oil between it and the cell walls. This cuticle bears the impressions of the cell walls upon which it originally rested; they are frequently visible under the high power. The cells of which the lower half of the gland consists can frequently be distinctly discerned.

Sketching.—Sketch two or three glands in different positions, as described on p. 36.

Contents.—Crush the glands by pressing gently on the coverslip; observe the granular mixture of oil and protoplasm that exudes. The presence of oil can be detected by the reagents mentioned on p. 37.

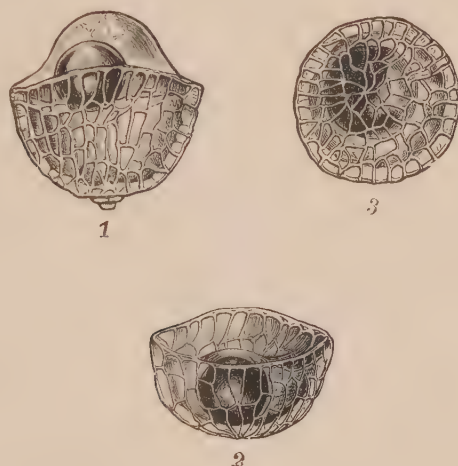


FIG. 28—Lupulin Glands. $\times 100$. 1 and 2, side views; 3, seen from below.
(Vogl.)

Adulterations.—Lupulin is not often adulterated, but it is generally very impure, containing sand, vegetable *débris*, &c. These are easily distinguished from the characteristic glands. Sand is usually in colourless fragments of irregular angular shape. Vegetable *débris* can be recognised by their cellular structure.

Note.—It is often necessary to prepare and examine several slides of lupulin before glands are found exhibiting their structure well. Too much pressure on the glands may be obviated by introducing a few splinters of a coverslip. Commercial lupulin is not well adapted for examination; the glands are usually dark in colour and much shrunken, hence their structure is not easily discerned.

Kamala

Source.—Kamala is a fine, granular, mobile powder of a dull red colour. It consists of the hairs and glands that cover the fruits of *Mallotus philippinensis*, Müll. Arg. Even to the naked eye it appears heterogeneous.

Mounting.—Mount a little kamala as directed for lupulin. Examine with the low power; groups of hairs and small garnet-red glands can be distinguished; particles of sand and of vegetable tissue are often also present.

Examination.—Examine the preparation under the high power; each group of hairs consists of a tuft of thick-walled,

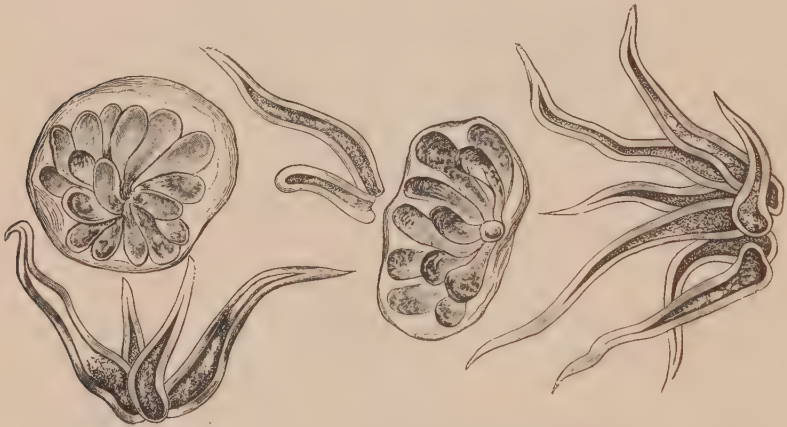


FIG. 29.—Kamala ($\times$ about 140), showing two glands with their secreting cells and three groups of hairs. (Moeller.)

short, pointed, divergent hairs which contain sometimes air, sometimes a granular substance, sometimes a reddish resin. Occasionally they are divided by delicate transverse walls into two or more cells. Observe that the hairs are seldom single. The glands are so deep in colour that their structure cannot be discerned and it becomes necessary to subject them to some suitable treatment.

Mount a fresh portion of kamala in solution of potash without previously moistening it with alcohol; the alkali readily dissolves the red resin, forming a deep brownish-red solution. Irrigate gently with solution of potash until the colour is almost completely removed.

Examine now with the low power, and, having selected a suitable gland, examine it more closely with the high power. It consists of a number of elongated cells radiating in a more or less regular manner from a common centre and enlarged at their free extremities. Observe that they are inclosed by a delicate membrane; this is the cuticle that has originally covered the secreting cells, but has been raised in the same way as the cuticle of the hop gland. The resin has been contained in the space between the cuticle and the cells, secretion having taken place on the radial as well as the outer wall of each cell.

Sketching.—Sketch, under the high power, a small group of hairs and two or three glands in different positions. Represent accurately the apex, the thickness of the wall, and the shape of each hair.

Adulterations.—Kamala is often grossly adulterated, but its characters are so well marked that it is easy to identify the hairs and glands of the genuine drug.

Vegetable *débris* often occur in it, but should not be present in excessive quantity. Minute fragments of reddish sand are frequently to be found in great numbers. Coloured starches have also been observed; these can be identified in the usual way, after decolourising them, if necessary, with alcohol.

The hairs and glands of *Flemingia sp.* (wars or wurrus) bear a general resemblance to kamala, but the hairs are usually single, and the glands contain secreting cells arranged in four or five tiers, instead of radiately.

SECTION IV

ERGOT

Source.—Ergot is the compact mycelium of *Claviceps purpurea*, Tulasne, developed in the inflorescence of *Secale cereale*, Linn.

Preparation.—Put a few well-developed ergots into a dish or beaker with a little wet blotting paper; cover the dish, and allow them to remain for a few hours. After exposure in this way to an atmosphere loaded with moisture the ergots will lose their rigidity and become flexible. In this condition they are well suited for cutting.

Most drugs are either so hard or so brittle as to require softening before sections can be cut from them. The most favourable condition is an almost tough or waxy one; neither so hard as to afford great resistance to the razor, nor so soft as to be flaccid. In many cases this can be most satisfactorily attained by exposing the drug to a moist atmosphere until it has acquired the desired condition. Such treatment is well adapted for soft drugs, such as leaves generally (very leathery ones excepted), many parenchymatous roots and rhizomes, &c. It is often preferable to soaking in water, which is liable to make soft drugs too soft and flaccid, and entails free contact of the various cell contents with water, which is often undesirable. Hard drugs, on the other hand, such as woods, woody barks, many roots, rhizomes, &c., require soaking, often prolonged for several days, in water or dilute glycerin. Others, again, require hardening in alcohol after soaking in water, as, for instance, cloves. No specific instructions, therefore, applicable to all cases can be given, but the aim should be to bring the drug into a waxy condition, if possible without soaking it in any liquid at all.

Embedding.—One of the ergots properly softened must next be embedded in elder-pith; this can usually be obtained from an optician or from one of the clockmakers in Clerkenwell. Select a piece about the thickness of the little finger and break off a couple of inches; lay it on the table, and halve it lengthwise. Each half should have the shape of a half-cylinder. Cut in each half a depression to receive the ergot; break the latter across the middle, and fix one half between the two pieces of elder-pith so that the transverse surface of the ergot is just below that of the elder-pith; bind them firmly in this position with a strong thread. The ergot is now ready for cutting.

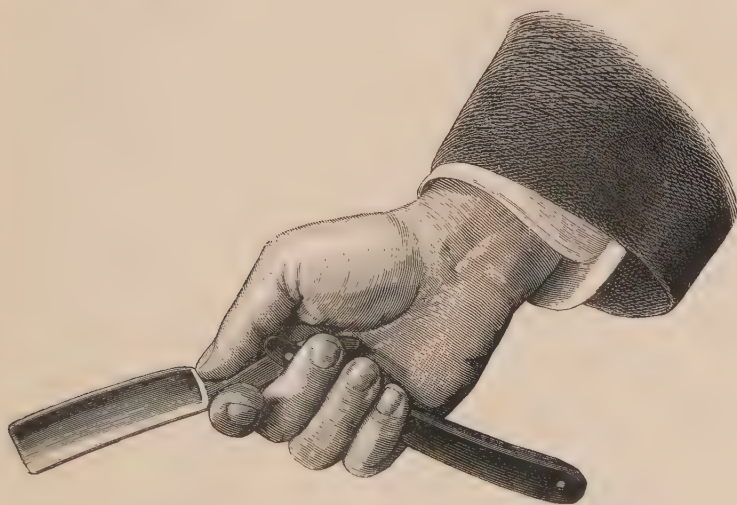


FIG. 30.—Showing the manner in which the razor should be grasped. (Behrens.)

Cutting Transverse Sections.—Bend the forefinger of the left hand and hold the elder-pith firmly but lightly between the thumb and the first knuckle-joint of the forefinger, the surface of the ergot being nearly level with the edge of the finger. Take the narrow steel tang of the razor between the thumb and forefinger of the right hand, so that the thumb is nearly parallel to the blade while the forefinger bends over it; the remaining three fingers then bend over the handle of the razor, and thus hold it securely but lightly.

Holding now the elder-pith and razor as directed, rest the flat part of the blade on the forefinger of the left hand, with the

heel of the blade near the pith ; then draw the razor from left to right, gently advancing it towards and through the pith so as to cut off a thin slice. Use the edge of the forefinger as a



FIG. 31.—Showing the relative positions of the razor, and the object in cutting sections by hand. (Behrens.)

guide for the razor blade, which should preferably have the side that rests upon the finger ground flat. Cut in this way about a dozen or more sections, transferring them from the blade of the razor to a small glass dish containing a little water.

Mounting and Examination.—Select one of the thinnest sections ; transfer it with a small brush to a drop of water on a slide ; cover with a coverslip and examine, first under the low and then under the high power. Observe the margin of the ergot, a narrow dark brown line which on closer investigation, especially where the section is thin, is seen to be a layer of small cells with dark brownish contents. Here and there are fragments of ill-defined tissue outside the dark line ; they are the remains of the outermost portion, which has perished. Within the brown line the cells show an oval, rounded, or elongated oval outline, the entire section consisting of such cells.

Contents.—These cells contain minute globules, and similar globules may be found scattered in the mounting medium near the section. They resemble oil globules. Apply the osmic acid test ; they gradually stain brown, showing that they consist, partly at least, of oil.

Mount a section in chloral hydrate ; the oil collects into

large globules. (The oil appears to exist intimately associated with proteid matter; this the chloral hydrate dissolves, setting free the oil in minute globules, which then easily unite into

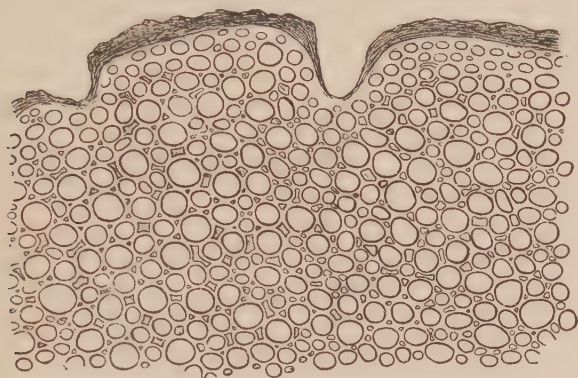


FIG. 32 Ergot of Rye. Transverse section, cleared. (Vogl.)

larger ones.) The cavities of the cells become more conspicuous.

Longitudinal Sections.—Cut now from an ergot by two transverse cuts a small piece about one-eighth of an inch long. Embed this in pith and cut longitudinal sections. Examine these. They present a structure very similar to that of the transverse section, but there are a greater number of cells with elongated oval outlines.

The ergot consists of a number of tubular cells (hyphæ) which interlace with one another and are so compact as to form a solid body. Although the cells run in all directions, there is a somewhat greater tendency for them to assume a longitudinal than a transverse position; hence in the longitudinal section the cavities are slightly more elongated. These cells contain chiefly fixed oil (osmic acid reaction) and the remains of protoplasm.

SECTION V

WOODS

INTRODUCTION

Objects of the Investigation.—The student should now proceed to the examination of more complex organs. In approaching this part of the subject he must bear in mind that the principal object sought is an accurate knowledge of the structure of the drug under examination. In the case of the woods, for example, his investigations should enable him to answer the following questions:

1. Of what elements the wood under examination is composed ;
2. What is the exact nature of these elements, the form of the cells, the markings on the walls, the material of which they consist ;
3. What are the contents of these elements (starch, calcium oxalate, resin, volatile oil, colouring matter, &c.) ;
4. How are these elements arranged ;
5. What are the particular characters of the elements present or their arrangement that would enable us to distinguish this drug from other similar ones.

From a medicinal point of view the woods can scarcely be called an important class of drugs, but the great variety of technical uses to which various woods have been put renders it very desirable for the student and analyst to be acquainted with the methods adopted in the examination of them and with the chief features by which one wood may be distinguished from another. The question of the identity of a wood is constantly arising, the problem to be solved being usually whether the wood used for a particular purpose is the wood it purports to be, or whether a cheaper and less valuable one has been substi-

tuted for it. Wood pulp is very largely used in the manufacture of paper, and consequently in the examination of paper the recognition of wood pulp becomes very necessary, even after it has undergone the process of bleaching by which the reactions yielded by the original wood are considerably modified. Wood may also be present in various surgical bandages, and is sometimes met with in powdered drugs, into which it may have been introduced either intentionally or accidentally.

Before proceeding to investigate the structure of medicinal woods the student should study the general structure of wood in his text-book of botany.¹

Definition.—By the term ‘wood’ botanists² understand all the secondary tissues produced by the cambium on its inner surface. This definition includes the softer, largely parenchymatous tissues that lie within the cambium ring of such a drug as calumba root, as well as the hard lignified tissues of which guaiacum, quassia wood, &c., are composed. In this section of the work, however, only those drugs that are grouped together by pharmacognosists under the term ‘ligna’ will be considered.

Typical Structural Elements.—The following are the chief types of elements that are met with in the woods, but it must not be forgotten that many intermediate forms occur.

- | | |
|---|---------------------|
| 1. Vessels. | 2. Tracheids. |
| 3. Wood fibres. | 4. Wood parenchyma. |
| 5. Cells of the secondary medullary rays. | |

1. *Vessels.*—These may usually be recognised by their comparatively large size, but they are more particularly characterised by the perforations in the transverse walls, by which a superposed row of cells has been converted into a vessel. The manipulations to which the tissues are subjected in separating the various elements from one another result in a separation of the vessel into the elements from which it was formed; these may be recognised by the perforations in the transverse walls (fig. 33, a).

The length of the vessels is not an important factor, but the width should be observed, although frequently vessels of

¹ Green, *Manual of Botany*, i. 340 et seq.; Vines, *Student's Text-Book of Botany*, p. 134 et seq., p. 196 et seq.

² Strasburger, Noll, Schenck, und Schimper, *Lehrbuch d. Botanik*, 1894, p. 103.

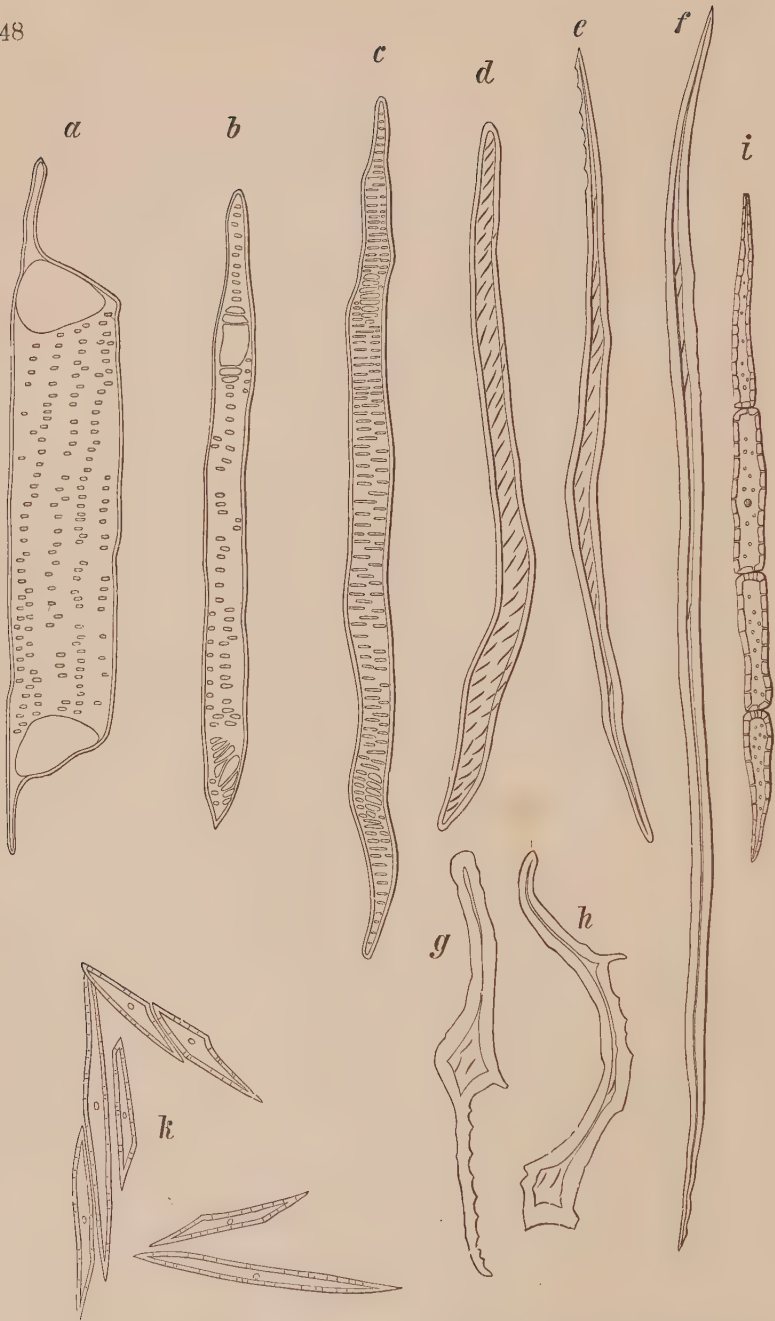


FIG. 33.—Elements of the Wood of the Copper Beech (*Fagus silvatica*).
a, vessel; *b*, intermediate form; *c* and *d*, tracheids; *e* and *f*, wood fibres;
g and *h*, wood fibres of irregular shape; *i*, wood parenchyma; *k*, cells
of medullary ray. (Schwarz.)

varying size are found in the same drug. Indeed the student should remember that absolute size is not always a very reliable character, as under different circumstances the absolute size may vary. Relative size, on the other hand, is more valuable, for the circumstances that increase the size of one element also increase that of the others, and the relative size therefore remains constant. More important than the size of the vessel are the size and character of the pits on its walls (fig. 34). These

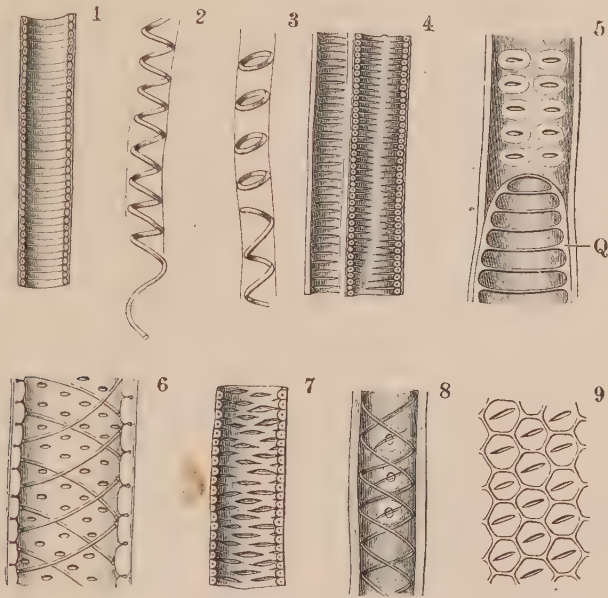


FIG. 34.—Various Forms of Vessels. 1 and 2, spiral; 3, annular, passing into spiral; 4, scalariform; 5, with bordered pits and scalariform perforation of oblique wall; 6, with simple pits and double spiral; 7, reticulated; 8, tracheid, with bordered pits and double spiral; 9, with bordered pits. (Vogl.)

may be large or small, round, angular, or elongated; they may be simple or bordered; all such details should be carefully noted.

The secondary wood never contains annular or spiral vessels.

2. *Tracheids*.—Tracheids are distinguished from vessels by their development from a single cambial cell, vessels being formed from a superposed row of cambial cells. They do not, therefore, as a rule, exhibit perforations by which superposed tracheids can communicate with one another (compare ipecacuanha wood, which is exceptional in this respect). They are

usually elongated, and have rounded or tapering but not sharply pointed ends (fig. 33, c). Their chief function being the transmission of water, they are commonly free from cell contents, but in forms intermediate between tracheids and wood parenchyma they may serve as storage cells for reserve material. From true wood parenchyma they are distinguished especially by their prosenchymatous form; from vessels by their smaller size and by the absence of perforations. Their walls usually bear small bordered pits, but in the tracheids of coniferous woods the pits are very large. In examining tracheids attention should be paid to the same points as mentioned in the previous paragraphs for vessels.

Cells that occupy a position intermediate between true tracheids and wood parenchyma have been called 'intermediate' or 'fibrous' cells. Like tracheids, each fibrous cell is developed from a single cambial cell, whereas several wood parenchyma cells are produced from a single cambial cell by transverse walls. They differ from true tracheids by being usually shorter and having more rounded ends, but particularly in serving as storage cells for reserve material. Fibrous cells may be thin-walled or thick-walled.

3. *Wood Fibres*.—These commonly form the principal constituent element of woods. They are usually long and narrow, and taper gradually at each end to a fine point (fig. 33, e and f). As their function is purely mechanical, they are destitute of protoplasmic contents, and do not act as storage cells for starch or other reserve material. They are developed from a single cambial cell, but their cavities are sometimes divided by very delicate transverse walls (chambered fibres). Their pits—which, as a rule, are not very numerous—are narrow, elongated slits, which may be simple or bordered, and are usually arranged in a left ascending spiral (left oblique). In determining the nature of the pits, care must be taken to examine longitudinal (radial or tangential) sections rather than the elements that have been separated from one another by digestion with nitric acid and potassium chlorate, or with chromic acid (see later), as after treatment with these reagents both the borders and the pits themselves are much less easy to see. In determining the direction of the pits it is necessary to be quite sure that the upper and not the lower wall of the cell is in focus, as one is very liable to focus through the cell to the lower

wall. The breadth of the cell and the thickness of the wall should also be noted, as these details sometimes form means by which the wood fibres of one drug may be distinguished from those of another. The length may also be of service, but not for identifying powdered woods, as the fibres are in that case mostly broken. Although true wood fibres never contain starch, they may nevertheless contain resin (guaiacum wood, red sanders wood) or volatile oil (yellow sandal wood), &c. The colour of the fibres and their contents may therefore be valuable indications of identity.

4. *Wood Parenchyma*.—Typical wood parenchyma cells are developed from a cambial cell by transverse division, and this origin can often be traced in the parenchymatous cells isolated from woods; very frequently a row of several superposed rectangular cells is terminated at both extremities by a bluntly conical one. Their walls are often moderately thick and freely pitted with simple pits. They often contain starch, calcium oxalate, &c.

The distribution of these cells, the thickness of their walls, the number, character, and distribution of the pits, as well as the nature of the contents, should be carefully determined.

5. *Cells of the Medullary Rays*.—When isolated these are scarcely to be distinguished from the cells of the wood parenchyma, but in sections or in such fragments as are often found in powdered woods they may be identified by their position and arrangement, for whereas wood parenchyma cells have their long axes parallel to the long axes of the wood fibres or vessels that accompany them, the long axes of the medullary ray cells are transverse to these.

Here also the same particulars should be studied as have been indicated for the wood parenchyma, and in addition the height and breadth of the medullary rays as exhibited by a tangential section.

Diagnostic Characters.—The principal diagnostic characters of woods are to be found in

- (a) The elements of which the wood consists, especially the presence of any element of unusual form, or the absence of any element of general occurrence; for instance, the absence of wood fibres and wood parenchyma (pine wood, &c.), presence of oil cells, ducts, &c.

- (b) The structural details of the elements present, especially the shape, thickness of wall, form and distribution of the pits.
- (c) The distribution of the tissues, especially the arrangement of the wood parenchyma, grouping and distribution of the vessels, width and height of the medullary rays.
- (d) The contents of these cells, particularly the presence of calcium oxalate and the crystalline form that it assumes, the presence of starch grains and the shapes they exhibit.
- (e) The size of the elements. In this respect it must be observed that the *absolute* size may vary according to the age of the trunk, but the *relative* size is always constant; hence, although the absolute size should be determined, greater weight is to be laid upon the relative size of each variety of cell present when compared with the others.

Although the examination and identification of powdered woods are by no means without interest or importance, the student is advised to refrain from that part of the subject until he has further advanced in his studies.

Quassia Wood

Source.—The quassia wood official in the British Pharmacopœia and used in this country is obtained from *Picræna excelsa*, Lindl. In Germany that obtained from *Quassia amara*, Linn., is also employed.

Quassia wood is one of the most suitable for the student to investigate on account of its relative softness and the ease with which it can be separated into its constituent elements.

Preparation for Cutting

From a small log of quassia wood saw off a disc about an inch thick. Split from this disc, by cuts passing through its centre, one or more wedge-shaped pieces, and remove the bark; the wood will present somewhat the appearance of fig. 35.

The surface of this piece of wood that is transverse to the

long axis of the log is the transverse surface, and any section taken from that surface or parallel to it is a transverse section (fig. 35, *tr*).

The surface exposed by cutting the disc downwards through the centre is a radial surface, because the cut is coincident with a radius of the disc. A section taken from this surface, or from any surface exposed by a radial cut (not by a cut parallel to the radius), is a radial section (fig. 35, *r*).

The surface exposed by cutting the disc downwards at right angles to the radius is a tangential surface, because the cut is

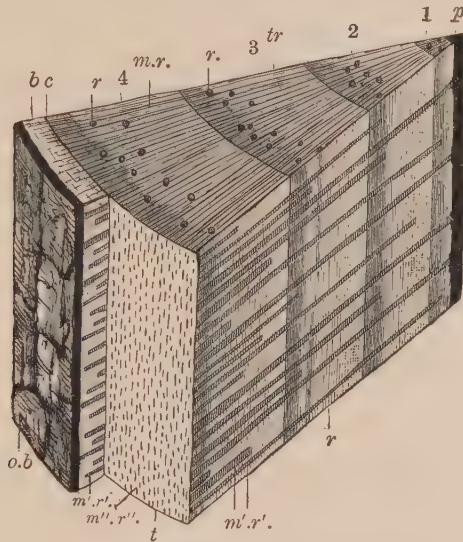


FIG. 35.—Piece cut from a pine stem four years old. 1, 2, 3, 4, the wood produced in the four successive years; *r*, *t*, *tr*, the radial, tangential, and transverse surfaces respectively; *b*, bark; *c*, cambium; *mr*, medullary ray on transverse section; *m'r*., the same on radial section both in wood and bark; *m'r'*., the same on tangential section; *r*, resin dust (on transverse section); *p*, pith. $\times 6$. (Strasburger, Noll, Schenck, and Schimper.)

made parallel to a tangent of the disc. A section made in this surface, or any surface parallel to it, is a tangential section (fig. 35, *t*).

Smooth the transverse surface with a sharp penknife, and examine it with a lens. Observe the medullary rays running in the direction of radii; they are sufficient to indicate the nature of the surface exposed (fig. 35, *m.r.*).

Do the same with a tangential surface, taking care, by ex-

amining the transverse surface, to see that the tangential surface is exactly at right angles to the medullary rays; if this be not the case, oblique sections will be obtained which are difficult of interpretation. Observe on the tangential surface small elongated-oval markings (fig. 35, *m'' . n''*.); these can be traced on the edge of the piece of wood, and can be identified with the medullary rays. Their particular appearance is sufficient to identify the surface as a tangential one.

Next cut a radial surface, taking great care that the cut passes as nearly as possible along a medullary ray; this can be done by comparing the transverse surface, and paring away the wood until the cut passes along a medullary ray. Examine now the radial surface, and observe that the medullary rays are evident as narrow bands, easily distinguishable from the remainder of the wood by their different appearance (fig. 35, *m' . n'*.).

The student should not on any account fail to make himself familiar with these three surfaces. Having done so, he can cut the piece of wood in varying oblique directions and examine the appearances of the sections. In cutting transverse, radial, or tangential sections of wood, he should also be very particular to have them as nearly exact as possible, as their interpretation is thereby much facilitated.

The term 'longitudinal' section is often employed to denote a section parallel to the long axis, and hence either radial or tangential. Since the term lacks precision, care should be taken in employing it.

Separation of the Elements

The student will find it most profitable to begin by separating the wood into its component structural elements and examining them.

From a radial surface cut with a penknife ten or twenty narrow thin slips or shavings, some about $\frac{1}{2}$ -inch long, $\frac{1}{8}$ inch wide, and $\frac{1}{32}$ inch thick, others thinner. Transfer them to a test-tube half full of nitric acid of specific gravity about 1.3 (two volumes of official nitric acid diluted with one of water answers very well); add to this about 20 grains of potassium chlorate in crystals, and warm gently until evolution of gas commences. The pieces of wood first darken in colour and

then bleach. Let the action continue, maintaining a gentle evolution of gas by warming, if necessary, from time to time, until glistening fibres are seen to be separated from the pieces of wood when the tube is gently shaken. Stop the action by carefully pouring the whole of the contents of the tube into a beaker of water. From this transfer the fragments of bleached wood by means of a glass rod to fresh water, and finally to

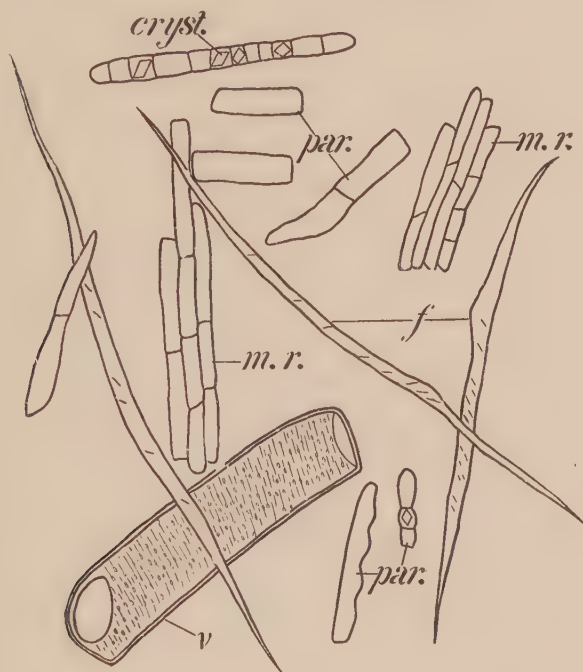


FIG. 36.—Quassia Wood. Elements isolated by potassium chlorate and nitric acid. *cryst.*, the membranes in which calcium oxalate crystals have been enclosed; *f*, wood fibres; *m. r.*, cells of medullary ray; *par.*, wood parenchyma; *v*, vessel. $\times 100$.

alcohol. In this they should remain a little time (if possible a few hours) to remove the gas that accumulates in the cells.

This process is known as Schulze's maceration process, and the mixture of nitric acid and chlorate of potash Schulze's maceration mixture (to be carefully distinguished from Schultz's solution, which is chlorzinciodine). The strength of nitric acid and the time necessary for disintegration vary with different drugs, and cannot, therefore, be definitely stated. If

the reaction is stopped too soon, the wood will not separate into its elements; if allowed to progress too far, the whole will be oxidised and destroyed.

Take one of the pieces of bleached wood from alcohol and transfer it to a drop of water on a slide; tease a small fragment with the dissecting needles until it separates into its component elements; cover and examine under the low power. Do not add glycerin, as it makes the cells too transparent.

Cells of the following forms will be found:

- (a) *Wood fibres* (fig. 36, *f*), conspicuous by reason of their length and tapering ends. Observe the width of the cell and the thickness of the wall.
- (b) *Wood parenchyma* (fig. 36, *par.*), recognisable by the oblong shape of the cells and the rounded pits in the walls. They often contain delicate membranes that present a quasi crystalline appearance; these are the membranes that have enveloped calcium oxalate crystals (see below). Sometimes several cells adhere end to end, the terminal ones being bluntly conical. Such a row of cells is developed from a single cambial cell by transverse division, and illustrates the formation of wood parenchyma.
- (c) *Cells of the medullary ray* (fig. 36, *m.r.*). These closely resemble the wood parenchyma; but they occur in plates of cells more often than in single rows, and when they adhere to wood fibres cross these at right angles; indeed, their position constitutes the sole means by which they can be distinguished from wood parenchyma.
- (d) *Vessels* (fig. 36, *v*). Although these are by no means so numerous as the wood fibres, wood parenchyma, or cells of the medullary rays, they are not difficult to find. They are readily identified by their much larger size, cylindrical shape, and numerous pits. On careful examination the remains of transverse walls with large perforations can often be distinguished.

The student should now sketch two or three of each kind of cell.

The most careful search will not disclose any other cell forms, and quassia wood is therefore built up of these elements. It remains to be seen how they are arranged.

Preparation and Examination of Sections

Radial Sections.—Take a well-soaked wedge of the wood. Smooth the transverse surface with a penknife, and examine it with a lens; make certain that the radial surface is exactly radial; if it is not, trim it with the knife until it is. Make on

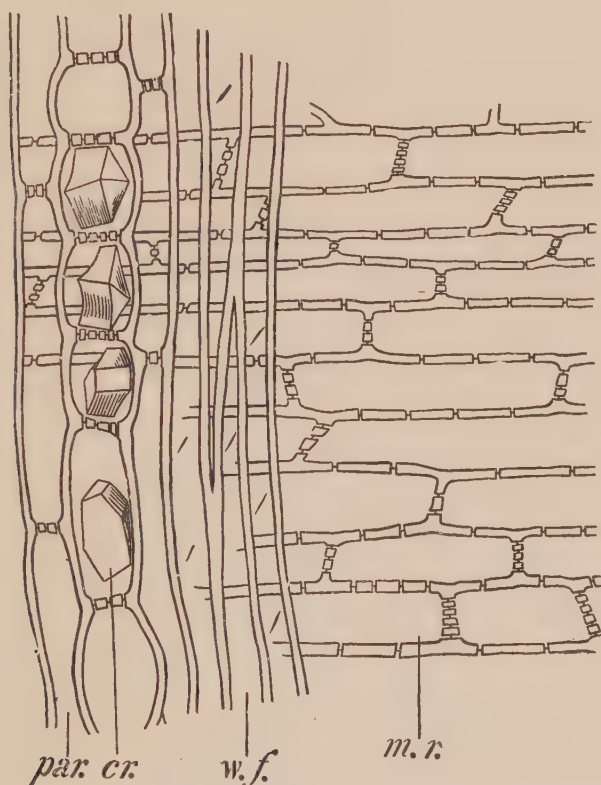


FIG. 37.—Quassia Wood. Radial section. *cr.*, crystals of calcium oxalate; *m.r.*, medullary ray; *par.*, wood parenchyma; *w.f.*, wood fibres. $\times 320$.

the radial surface a pencil mark about $\frac{1}{8}$ inch square, or rather less. Split the wedge, and cut out the pencil mark to a depth of $\frac{1}{8}$ inch so as to obtain a rectangular fragment, the small end of which bears the pencil mark. Fix this fragment in pith, so that the pencil mark is level with the surface. Cut several sections, rejecting the first two or three. Be careful to draw

the edge of the razor, from heel to point, across the wood, and avoid any motion similar to that used in sharpening a pencil.

Transfer the sections, as cut, to spirit, in which they should remain for several minutes; mount one in dilute glycerin (equal parts), taking care to spread the section out with the needles if necessary. Cover and examine. If the section contains many air bubbles, warm it until the liquid gently boils, and cool.

Identify on this section (or others if necessary) all the elements that have been previously observed and sketched.

The *medullary rays* will be conspicuous as broad bands of cells extending along the entire section, provided that it has been truly radial; most of these cells are elongated in the direction of the radius.

The *wood fibres* are easily found. They are long and narrow, and assume a direction at right angles to the medullary rays. The tapering ends are not easy to see, as the fibres interlace; hence the dissociation treatment is valuable, as it is the only means by which the shape of the fibre can be accurately ascertained.

The cells of the *wood parenchyma* are axially elongated; they are not so numerous as the wood fibres; from the cells of the medullary rays they are distinguished by their axial (not radial) elongation.

The *vessels* are very large, but only a portion of the pitted wall can, as a rule, be found; large spaces between the wood fibres indicate the position of the vessels, the walls of which are often cut away.

Observe in the cells of medullary rays and wood-parenchyma large crystals of calcium oxalate.¹ They are enclosed in delicate membranes, which are invisible until the crystal is dissolved away, when they are left behind (compare the examination of the dissociated cells).

Observe also occasional small grains of starch (iodine reaction).

Sketch a small portion of the radial section, taking care to include part of the medullary ray.

Tangential Sections. In the same way prepare a piece of wood for tangential section, taking care, by examining

¹ It must be observed that in some specimens of Jamaica quassia wood calcium oxalate crystals are difficult to find.

with a lens, that the surface marked is really the tangential surface.

Observe in this section wood fibres, wood parenchyma, and vessels presenting the same appearance that they did in radial section. The medullary rays, on the other hand, are cut trans-

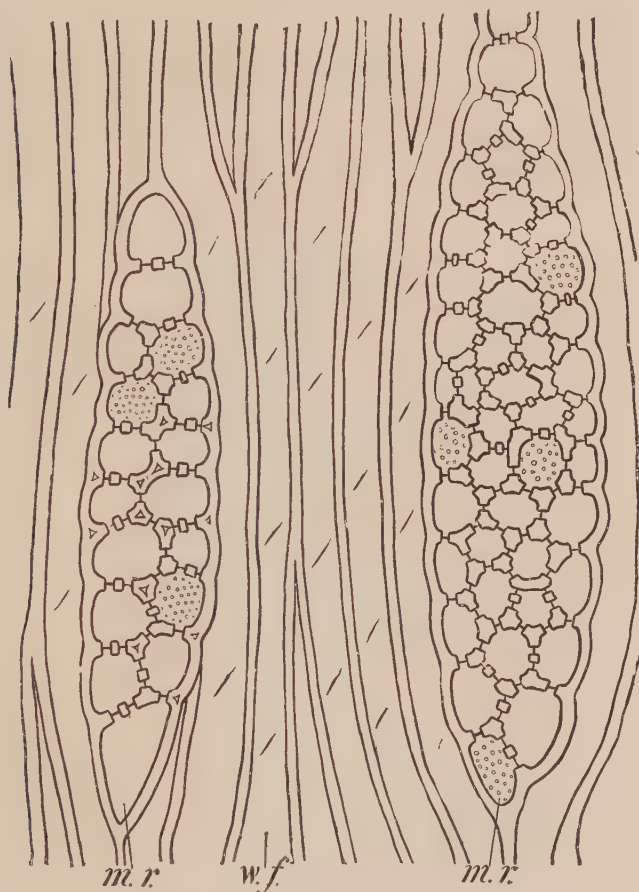


FIG. 38.—Quassia Wood, tangential section. *m.r.*, medullary rays; *w.f.*, wood fibres (the pits in the walls of the cells of the medullary rays are rather too conspicuous). $\times 320$.

versely to their length, whereas in the radial section they are cut parallel to their length. The tangential section, therefore, exhibits the height and breadth of the medullary rays in the form of elongated oval groups of cells inserted between the wood fibres. Count the average number of cells in the length and greatest

breadth of the rays. Beyond this do not examine the section further.

Sketch a portion of the section.

Transverse Sections.—Lastly, cut in a similar manner transverse sections. Examine first the medullary rays. These exhibit their length, but the breadth is seen only at the particular point at which they happen to have been cut (compare the tangential section); hence they are mostly two or three cells wide, but occasionally as many as four (middle of a large ray) or as few as one (upper or lower extremity of a ray). Observe that the cells appear radially elongated.

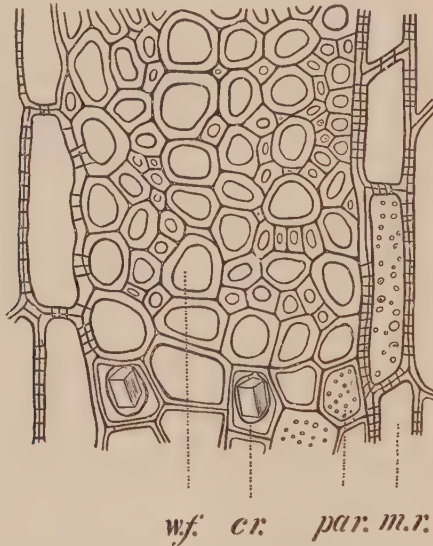


FIG. 39.—Quassia Wood, transverse section. *cr.*, crystals of calcium oxalate, enclosed in delicate membranes; *m.r.*, medullary ray; *par.*, parenchyma of wood; *w.f.*, wood fibres. $\times 320$. (After Meyer.)

Next examine the vessels. Their large size makes them conspicuous. They are usually in small groups of two or three, often extending from one medullary ray to the next.

The remainder of the wood must be composed of wood fibres and wood parenchyma, since no other elements are present. These are at first not very easily distinguished from one another.

The wood fibres have thick walls; the cells are polygonal in outline, often varying much in size according to the point in

their length at which they have been cut ; they do not exhibit pits nor any transverse walls with pits.

The wood parenchyma cells have comparatively thin walls, are of more uniform size, and more regularly rectangular ; they often exhibit pits, and sometimes crystals. They occur mostly in groups stretching from ray to ray, and often form irregular rings on the section of the trunk (false annual rings).

Sketch a small portion of the section.

Take a fresh section, transfer it to a drop of water on a slide ; remove the water by filter paper, add one or two drops of solution of phloroglucin, and cover with a watch-glass. After two or three minutes (longer for delicate experiments) remove the phloroglucin solution by filter paper, add a drop of strong hydrochloric acid, cover with a coverslip, and examine.

All the cell walls should have assumed a deep crimson red colour, an indication that they are lignified. The calcium oxalate is dissolved by the acid ; the membranes in which the crystals were enclosed remain, and in favourable sections (tangential or radial) may be seen also to be lignified.

The student should now sum up the results of his investigation as follows :

- (1) The only elements present are wood fibres, wood parenchyma, vessels and cells of medullary rays.
- (2) The medullary rays are four to twenty cells high and one to four cells wide ; they are mostly ten cells high and three cells wide. The vessels are sometimes single, more often in groups of two or three ; the wood parenchyma is mostly in bands extending from ray to ray.
- (3) The cell walls are all lignified. The wood parenchyma and medullary rays contain calcium oxalate in single crystals, and, here and there, a little starch.
- (4) The wood fibres are long and taper gradually to fine points ; they have moderately thick walls.

A comparison of the anatomical structure of the official quassia wood with that of Surinam quassia will illustrate the value of these characters. Surinam quassia has medullary rays that are mostly one cell wide, and is free from calcium oxalate ; the official (Jamaica) quassia has medullary rays from one to four cells wide, and contains calcium oxalate.

Other Methods for separating Tissues into their Elements

Although Schulze's maceration mixture is the method usually adopted, it is by no means the only one, nor is it in all cases the best.

Isolation by Chromic Acid.—A solution of 10 per cent. of chromic acid in dilute sulphuric acid forms a very useful oxidising mixture capable of destroying the middle lamella and thus isolating the cells. It is best used as follows:

Cut several sections (preferably radial and not too thin) and immerse them in a watch-glass containing twenty to thirty drops of the solution. From time to time transfer a section with a glass rod to a drop of water on a slide, wash with one or two drops of water, and tease it a little with the needles or press it with a glass rod. As soon as the trial section easily separates into its elements transfer the remaining sections carefully to a dish of water. Mount one in a small drop of water and cover with a coverslip; a little pressure on the coverslip accompanied by a sliding movement will bring about the separation of the cells.

The time required varies with the object and temperature; from ten to thirty minutes often suffice.

Isolation by Mangin's Method.—Mangin's method of separating the cells is based upon his belief that the middle lamella consists of calcium pectate. The sections are first digested for forty-eight hours in a mixture of alcohol 4 volumes and hydrochloric acid 1 volume; this dissolves the calcium as chloride, leaving the pectic acid undissolved. The sections are then digested in a 10 per cent. solution of ammonia or ammonium oxalate, which removes the pectic acid as pectate. The middle lamella having been thus dissolved, the cells separate.

This method is not so suitable for woods as either the nitric acid or chromic acid method.

Richter's Method.—Richter has shown that a strong solution of ammonia answers a similar purpose, but it requires a much longer time (14 days) and is more suitable for parenchymatous tissue than for wood.

Vétillard's Method.—This depends upon the action of boiling 10 per cent. solution of sodium carbonate, and has

already been described in Section II. It is specially suitable for the purpose there indicated.

Digestion with Potash.—Isolation by caustic potash is particularly well adapted for parenchymatous tissues (leaves, &c.) but not for wood. Fragments of the objects are digested in water containing from 1 to 5 per cent. of caustic potash on a water-bath for ten to thirty minutes; they are then washed free from alkali with distilled water, and the cells separated by teasing with the needles. The treatment with caustic alkali is liable to produce swelling of the cell wall, which has always to be taken into consideration.

Putrefaction.—By this means the layers of cells in the pericarp of the wheat and other grains can be loosened and separated. The grains are crushed, mixed with water, and set aside in a warm place (20–30° C.) for several days. The layers are then separated by teasing and stripping.

Tests for Lignification

Phloroglucin.—The best test to detect lignification of the cell wall is that by phloroglucin and hydrochloric acid, as described on p. 61. The following, however, are useful, and the student should make himself familiar with them.

Chlorzinciodine.—This reagent is best applied by mounting a section in water (without the coverslip), removing the water as completely as possible by filter paper, dropping a drop of the reagent upon the section, covering, and examining. While cellulose assumes a bluish or violet colour with the reagent, the lignified cell wall is stained yellow. If, however, the extent of the lignification is slight, the yellow colour may be overpowered by the blue cellulose reaction.

Iodine and Sulphuric Acid.—The mode of using these reagents has already been described (p. 25); the lignified cell wall assumes a yellow colour.

Aniline Hydrochloride, with or without hydrochloric acid, colours the lignified wall yellow, and is useful when the presence of acid is objectionable.

The substances that produce these colour reactions are destroyed by strong oxidising agents, and are removed by prolonged digestion with caustic alkalis; hence the isolated elements after maceration with nitric acid and potassium

chlorate or with caustic potash may not give the lignin reaction. Many other reagents for the detection of lignification of the cell wall have been proposed, but none of them have come into such general use as the phloroglucin test.¹

Removal of Air from Sections

Air bubbles frequently occur in microscopical preparations, sometimes in small, sometimes in large numbers. In the former case they seldom materially interfere with the examination of the preparation, but in the latter their removal is often necessary. They may be recognised by the wide black margin surrounding a brilliant centre. When lying free in the mounting medium they appear circular, but in cells they usually fill the cavity and assume the outline of the cell.

One of the following methods may be adopted for removing them :

- (1) Transfer the sections to strong alcohol, and allow them to remain in it until freed from air ; the time necessary for this varies from fifteen minutes to twelve hours. The method is usually successful, but takes time, and has further the disadvantage that all cell contents soluble in alcohol, such as volatile oil, resin, &c., will be dissolved, and possibly other changes induced. If the structure only of a drug is to be studied, this is seldom objectionable, but for the examination of the cell contents other means of attaining the object must be adopted.
- (2) Heat the sections in water, dilute glycerin, or other medium until the liquid boils ; keep it gently boiling for a few seconds, then cool. This method also entails alteration of the cell contents, and not unfrequently alteration of the cell wall, which has a tendency to swell under these conditions. It is, however, effectual and often resorted to, especially for sections of hard tissues, which may be thus treated on the slide (covered with a coverslip).
- (3) Place the sections in a dish of recently boiled and cooled distilled water ; allow them to remain until the water

¹ For what is known of the nature of lignin compare Cröss and Bevan (*Cellulose*, 1895, p. 94).

has dissolved the air, for which usually several hours are necessary. The method is very effectual, and entails but little change in cell walls or cell contents, hence it is in many cases preferable to the first two methods. Care must be taken to boil the water for ten minutes, to cool it thoroughly, and to use it in abundance, say from 25 to 50 cubic centimetres for a dozen sections.

- (4) Place the sections in a small dish of water under the receiver of an air-pump and exhaust the air. By this means the air is rapidly and effectually removed from the sections; but unfortunately an air-exhaust is not always at hand, otherwise this would probably prove the best and easiest means of getting rid of air.

The student should be careful to bear in mind the disadvantages of the various methods enumerated, and to select that method which will best answer for the particular section to be treated.

Guaiacum Wood

Source.—The official guaiacum wood is the heart wood of *Guaiacum officinale*, Linn., and of *G. sanctum*, Linn.

Preparation.—On account of its extreme hardness guaiacum wood is not easy to cut; it requires prolonged soaking in water or dilute glycerin. The structure may be equally well determined by the examination of the pale sapwood, which is much softer, but in this case the localisation of the resin cannot be ascertained.

The sections may be examined in glycerin. The elements may be separated by macerating radial sections in chromic acid.

Description.—The transverse section shows that the bulk of the wood consists of very strongly thickened wood fibres, which are often cut more or less obliquely, due to the varying course which they take in the stem. In section they appear polygonal or nearly rounded. Isolated by chromic acid (or other means) they are seen to be mostly of moderate length (400 to 600 μ) and very thick-walled; they either taper gradually to a fine point or are abruptly narrowed towards the end, not unfrequently forking at or near the extremity. The pits are scattered clefts.

The vessels are mostly large and isolated, seldom two or three together. They have thick, pitted walls, and are often filled with yellow resin. In surface view the walls are seen to bear very numerous small pits.

The wood parenchyma cells are arranged in narrow tangential bands, usually one cell wide, but sometimes two or three ;

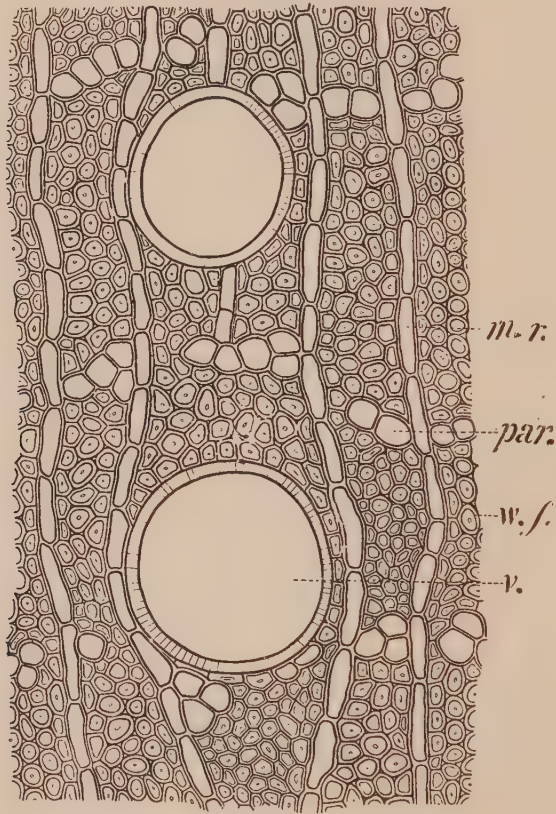


FIG. 40.—Guaiacum Wood, transverse section. *m.r.*, medullary ray; *par.*, wood parenchyma; *v.*, vessel; *w.f.*, wood fibres. $\times 320$. (After Berg.)

such bands often stretch continuously from one medullary ray to the next, and so form false annual rings. In these cells calcium oxalate crystals are by no means uncommon.

The medullary rays are one cell wide and (in tangential section) from four to six cells high; they often assume a

sinuous course, deviating to allow room for the vessels. The cells are pitted, and are strongly radially elongated.

In the heart wood, the vessels, wood parenchyma, and medullary rays are filled with yellow or brown resin, which also seems to permeate the walls of the wood fibres and even be present in the cavity. In the cells of the wood parenchyma a large crystal of calcium oxalate can be found here and there.



FIG. 41.—Yellow Sandal Wood, transverse section. $\times 200$. (Petersen.)

Yellow Sandal Wood

Source.—Yellow sandal wood is the heart wood of *Santalum album*, Linn.

Description.—The medullary rays are from one to three, often two, cells wide and about six high; the cells are strongly radially elongated, and have large pits.

The wood consists principally of thick-walled wood fibres. The vessels are mostly isolated and large, varying from 50 to 80 μ in diameter. The wood parenchyma is small in quantity;

it is distributed in tangential or oblique groups of from two to five cells, not often long enough to extend from one medullary ray to the next. Here and there a large crystal of calcium oxalate can be seen; in radial or tangential sections axially extended rows of from ten to fifteen such crystal cells can be found.

Thin longitudinal slices (or rather thick sections) can readily be separated into their constituent elements by maceration in chromic acid solution. After replacing the reagent by water the elements can be examined. Globules of oil (which stain

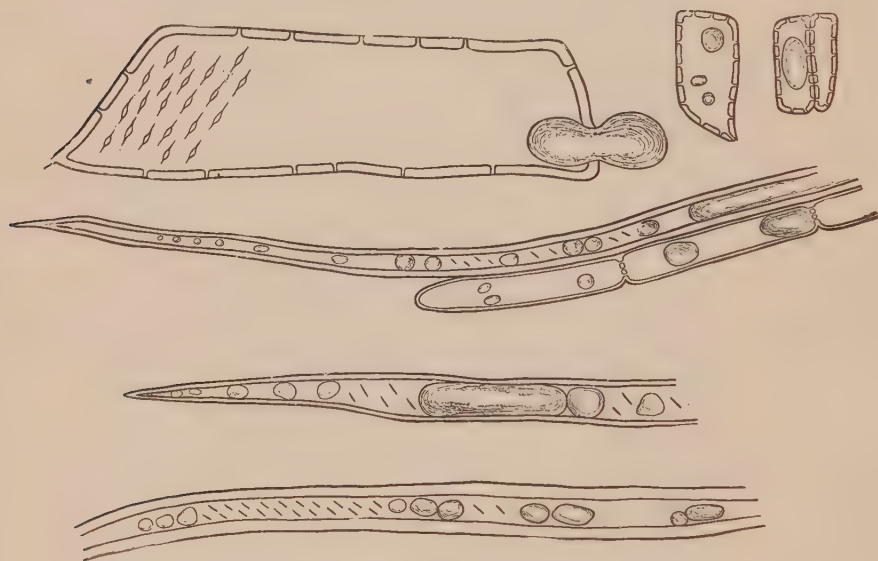


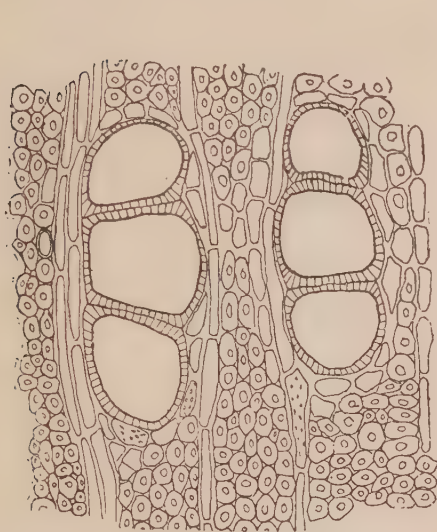
FIG. 42.—Yellow Sandal Wood. Elements isolated by chromic acid and showing the volatile oil (in the vessel some of the pits only have been introduced). $\times 300$.

with alkanna or osmic acid) are to be seen in all of them, but the wood parenchyma and medullary rays appear to contain most. The oil dissolves readily in alcohol (distinction from most fixed oils), and is doubtless the volatile oil that imparts to the wood its characteristic aroma. It is remarkable that in this case the oil is not secreted in special cells, but found in all the constituent elements of the wood.

The wood of *Fusanus acuminatus*, R. Br. (South Australia), is distinguished from that of *Santalum album* by the arrangement of the vessels; these mostly form radially extended groups of

from two to five. The wood contains but little calcium oxalate, and in tangential section the medullary rays often exhibit an elongated terminal cell which adjoins a similar cell belonging to a ray above or below.¹

The wood of *Amyris balsamifera*, Linn. (Venezuelan Sandal Wood) exhibits vessels arranged in long radial rows, and both these and the wood parenchyma contain a yellow resin, but only a little volatile oil can be detected by osmic acid.



43.—Wood of *Fusanus acuminatus*, transverse section. $\times 200$. (Petersen.)

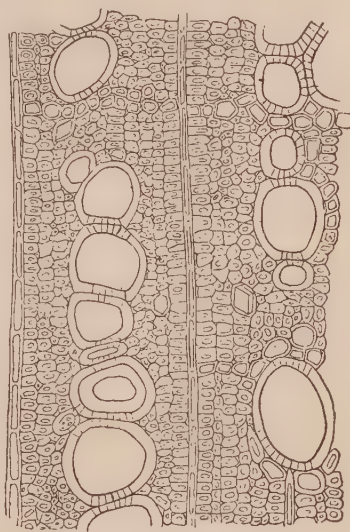


FIG. 44.—Venezuelan Sandal Wood, transverse section. $\times 200$. (Petersen.)

The student should compare figs. 43 and 44 with fig. 41, and observe attentively the differences in structure which enable these woods to be easily distinguished from one another.

Pine Wood

From a fragment of pine wood (ordinary firewood will do) cut radial sections; transfer them to alcohol; mount, and examine one in dilute glycerin.

¹ Petersen, *Pharm. Journal* [3], xvi. 757.

Observe the large bordered pits on the radial walls of the tracheids.¹

Cut and examine tangential sections; the large pits previously seen in surface view are now mostly seen in section, the tangential walls bearing smaller pits. Observe particularly the section of the pit (compare the section of a bordered pit in the text-book of botany).

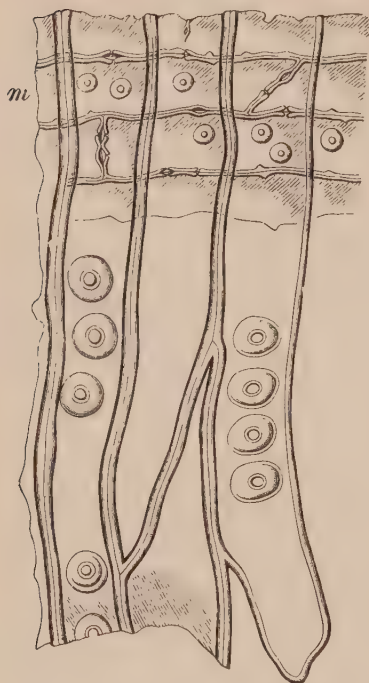


FIG. 45.—Bordered Pits of Fir.
m, medullary ray. (Tschirch.)

Disintegrate a little of the wood by maceration with potassium chlorate and nitric acid; with the exception of the medullary rays, the wood consists entirely of tracheids.

From a little pine sawdust sift some of the finest powder. Examine in dilute glycerin. The pits are easily seen and identified; some present their surface view, some their section.

The student should not fail to make himself familiar with the microscopical characters of sawdust, as it is

often found in small quantity in vegetable powders, not necessarily, however, as an adulteration.

Calcium Oxalate

Calcium oxalate is of very frequent occurrence in foods, drugs, and vegetable substances generally. Being practically insoluble in water, or in the feebly acid cell sap, it is met with

¹ In the illustration similar bordered pits are seen on the cells of the medullary ray. That is the case with the outer rows of cells of the medullary ray of the fir, but the spruce fir has simple pits and the cells are all alike; in the pine there are two kinds of cells, the outer being distinguished by the very irregular jagged outline of the secondary thickenings.

in the solid and more or less definitely crystalline form. It exhibits a considerable variety, both in the shape and size that the crystals assume, but these remain fairly constant for one and the same plant, and not unfrequently genera or even natural orders are characterised by the more or less regular occurrence of calcium oxalate crystals of particular form. It acquires, therefore, high importance as a valuable means of identifying a drug. During the process of pulverisation to which drugs are often subjected, these crystals, especially if they are of small size, remain in large part intact, and serve therefore to characterise the powder just as they characterise the entire drug. Large crystals are more or less broken during pulverisation, but even in this case there is little difficulty in detecting the broken fragments and recognising in them portions of larger crystals.

Although calcium oxalate is one of the most frequent of the crystalline contents of the cell, it is by no means the only one, and the student must be careful to determine the nature of any crystals that may be found before reporting them as calcium oxalate. This is particularly necessary when examining an unknown drug.

The following are the chief reactions that characterise this compound:

- (1) It is insoluble in acetic acid, but soluble in hydrochloric acid without effervescence (distinction from calcium carbonate).
- (2) In contact with concentrated or moderately concentrated sulphuric acid it is decomposed; the oxalate disappears, while acicular crystals of calcium sulphate make their appearance in the immediate neighbourhood or at a little distance, according to the current in the mounting medium at the moment when the reaction took place.
- (3) Solution of barium chloride does not react with them (distinction from calcium sulphate, which is occasionally found; crystals of this substance would become encrusted with barium sulphate).

The student should, however, remember that many reagents attack calcium oxalate, especially if the latter is present in comparatively small quantity and the temperature

is elevated. Thus the digestion with solution of caustic potash, which is often resorted to for separating the elements of vegetable tissues, may produce a marked effect on the crystals of calcium oxalate, partially dissolving them, with the production, doubtless, of potassium oxalate and calcium hydrate. Prolonged heating with solution of chloral hydrate often produces a similar effect.

Calcium oxalate is a dimorphic salt. It crystallises either with three molecules of water in crystals belonging to the

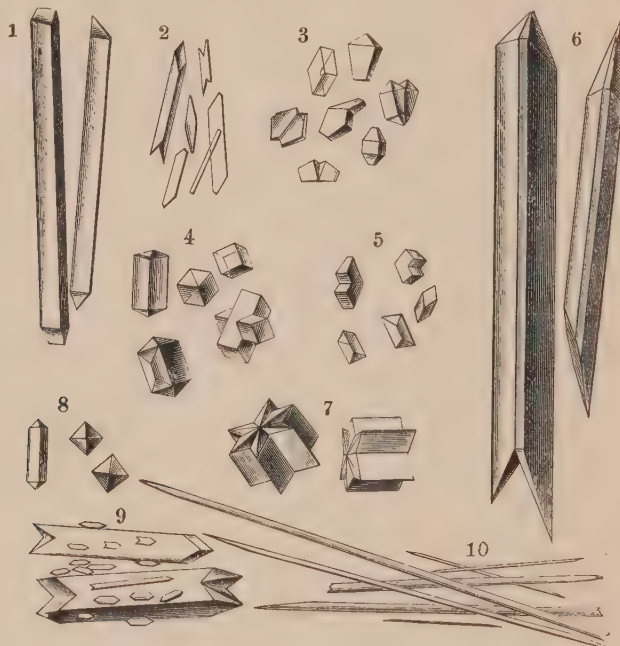


FIG. 46.—Various Forms of Calcium Oxalate Crystals. 1, from cusparia bark; 2, rhatany root; 3, liquorice root; 4, henbane leaves; 5, quebracho bark; 6, orris rhizome; 7, leaf of *Pavonia* sp.; 8, leaf of *Convolvulus arvensis*; 9, quillaja bark; 10, squill bulb. $\times 250$. (Vogl.)

tetragonal system, or with one molecule in crystals belonging to the monoclinic system. To these fundamental differences in the composition and crystalline form must be added differences that are produced by other influences at present not well understood, as, for instance, the rapidity of the currents in the cell sap, the degree of acidity of the latter, the relative amount of calcium present, and so on. These causes all tend to produce a great diversity in the shape, size, &c. of the

crystals that are formed, and the latter acquire, therefore, a high diagnostic value. As the great majority of plants contain calcium oxalate in some form or other, its absence (foxglove leaves, lobelia herb) is also a valuable indication of identity.

The forms assumed by the crystals of calcium oxalate may for convenience be classified as follows:

Single crystals;

Aggregates of crystals (rosettes); *Rhume, Shio*

Sphæro-crystals (very rare); *H*

Groups of acicular crystals (raphides); *M*

Sandy crystals. *Belladonna*

Single crystals are exceedingly common. They may occur singly, or two or more together in the same cell. They may

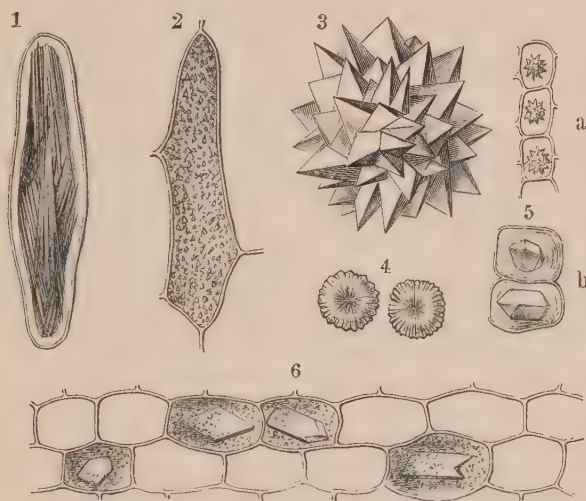


FIG. 47.—Various Forms of Calcium Oxalate Crystals. 1, raphides; 2, sandy crystals (belladonna root); 3, rosette or cluster crystal (rhubarb rhizome); 4, rosettes (argel leaves); 5, a, rosettes; b, single crystals (horse-chestnut bark); 6, single crystal embedded in sandy crystals. $\times 160$. (Vogl.)

belong to the tetragonal or to the monoclinic system, although the latter are more common. Not unfrequently twin crystals are formed.

Raphides are bundles of long acicular crystals belonging to the monoclinic system. They are very common in monocotyledonous plants, and are often embedded in mucilage. Very frequently the cells containing these crystals are arranged in axial rows.

Rosettes of calcium oxalate are very widely distributed. They vary considerably, both in size and appearance. Apparently they may belong either to the quadratic or monoclinic system.

Sphæro-crystals are closely allied to the rosettes, but their occurrence is rare.

Sandy crystals are very common in Solanaceæ and some other natural orders. The crystals are so minute that their crystalline form is not easily ascertained; they appear, however, to belong to the monoclinic system. Great numbers of these crystals occur in a single cell, completely filling it, and appearing, for optical reasons, as a dark patch that might be mistaken for dust or dirt.

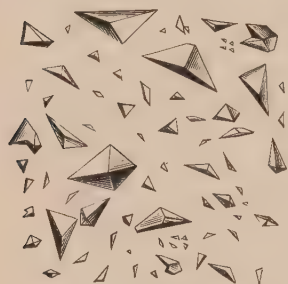


FIG. 48.—Sandy Crystals.
× 450. (Vogl.)

Single crystals and rosettes are frequently found surrounded by a membrane of lignified cellulose. This membrane retains the shape of the

crystal, even after the latter has been dissolved and removed, and it can be stained by suitable reagents. Sometimes the membrane is easily seen, but sometimes it is so thin as to be invisible until stained.

SECTION VI

STEMS

INTRODUCTION

UNDER this heading the structure of the stems of some of the official herbs will be considered. These consist of a ring of wood enclosing a pith and surrounded by the bast, cortex, and epidermis. In addition, therefore, to the elements of the wood, these organs will contain epidermis (or possibly the cork that has taken its place), cortex, bast, and pith.

The epidermis usually consists of a single row of cells that vary considerably in size and shape, though they commonly exhibit tangential elongation in transverse sections and axial elongation in longitudinal sections. It generally bears stomata as well as hairs of varying nature, both simple (protective) and secreting (glandular), and is covered with a cuticle. The characters of this tissue will be more fully described in Section VII., and the foregoing remarks may, therefore, suffice for the present.

Following upon the epidermis is the cortex, the inner limit of which is the endodermis. The cortex is generally composed of parenchymatous cells which in transverse section are tangentially elongated near the epidermis, but become more or less isodiametric near the endodermis, and generally exhibit intercellular spaces. That part that abuts upon the epidermis is often collenchymatous—that is to say, the cells exhibit a considerable thickening, especially at the angles; such collenchymatous tissue, however, seldom assumes the character of true typical collenchyma, in which the cells are thickened only at the angles.

If the leaves are small, and the stem takes over, in part at least, the functions of the leaves, then palisade tissue may be developed below the epidermis.

Although the parenchymatous cells of the cortex do not usually exhibit much variation in different plants, yet this tissue often contains other cell forms or special cell contents that may materially assist in the diagnosis of a drug or its powder. Among such the following may be noted as of frequent occurrence :

I. Cell forms—

- | | |
|------------------------------|---------------------------|
| (a) Palisade. | (e) Oil cells. |
| (b) Sclerenchymatous cells. | (f) Oil glands. |
| (c) Sclerenchymatous fibres. | (g) Laticiferous cells. |
| (d) Internal hairs. | (j) Laticiferous vessels. |

II. Cell contents—

- | | |
|------------------------|--------------------------|
| (a) Chlorophyll | (e) Tannin, &c. |
| (b) Calcium oxalate. | (f) Oil, &c., in special |
| (c) Calcium carbonate. | cells or tissues. |
| (d) Starch. | |

These various cells and cell contents will be noticed in detail in Section VII. For the present the student should confine his attention to such as present themselves in the drugs examined.

In many stems, especially young stems, the endodermis can be distinguished ; it may be identified by the following characters :

- (a) The shape of the cells ; in transverse section they are usually rectangular, strongly tangentially elongated, and exhibit no intercellular spaces.
- (b) The nature of the walls ; these are usually thin, but they are commonly suberised, and often lignified ; this is especially the case with the central portion of the radial wall, which usually exhibits a slight local thickening, which is lignified.
- (c) The contents ; the cells of the endodermis are sometimes distinguished by containing an unusual quantity of starch, &c.

Sometimes, however, the endodermis cannot be identified.

The tissue immediately within the endodermis and abutting directly on it is the pericycle. This may consist of one or more rows of cells, which may remain parenchymatous, or may

develop, wholly or partially, into sclerenchymatous cells or fibres; the latter are especially common, and are often designated primary bast fibres. They may occur singly or in groups, or they may form a continuous ring.

Next to the pericycle is the bast ring.

As the primary bast is usually with difficulty to be distinguished and is not important for present purposes, it may be neglected, and the bast ring may be said to consist of bast rays and medullary rays.

This tissue is very small in the official herbaceous stems, and is of little diagnostic value except in so far as it may contain bast fibres, sclerenchymatous cells, or secreting tissue.

The wood may, if necessary, be examined as detailed in Section V. It is desirable here to note that a few abnormally developed woods may contain groups of bast tissue (bast parenchyma and sieve tubes) isolated in the wood. These are termed 'interxylary' bast. The following are the chief natural orders in which this abnormal development occurs.¹

Vochysiaceæ, Combretaceæ, Myrtaceæ, Melastomaceæ, Lythrarieæ, Onagrariæ, Cucurbitaceæ, Apocynaceæ, Asclepiadeæ, Loganiaceæ, Gentianeæ, Convolvulaceæ, Solanaceæ, Acanthaceæ, Euphorbiaceæ.

The pith consists of parenchymatous cells frequently of considerable size, often lignified, and usually thin-walled. It may contain sclerenchymatous fibres, sclerenchymatous cells, or the various forms of secreting tissue.

In a number of natural orders groups of bast tissue, occasionally accompanied by fibres, are found on the outer margin of the pith and abutting on the wood. These are known as 'intraxylary' or 'perimedullary' bast. The following are the chief natural orders in which this tissue has been observed: ¹

Vochysiaceæ, Malpighiaceæ, Olacineæ, Leguminosæ (Papilionaceæ), Combretaceæ, Melastomaceæ, Lythrarieæ, Cucurbitaceæ, Apocynaceæ, Asclepiadeæ, Loganiaceæ, Gentianeæ, Convolvulaceæ, Solanaceæ, Acanthaceæ, Thymelæaceæ, Euphorbiaceæ.

Diagnostic characters of herbaceous stems are to be sought chiefly in the following particulars:

- (1) Epidermis; for details of the particular features of this tissue compare the following section (Leaves).

¹ For complete lists see Solereder, *Anatomie der Dicotyledonen*.

- (2) Primary cortex and bast ; the cell contents, such as calcium oxalate, &c., presence and nature of any secretory tissue, presence of sclerenchymatous fibres.
- (3) Wood ; compare the preceding section, note the presence or absence of interxylary bast, or any other abnormal structure.
- (4) Pith ; nature and contents of cells ; presence or absence of intraxylary bast.

Lobelia Stem

Source.—Lobelia is the herb *Lobelia inflata*, Linn., cut while in flower and dried.

Preparation of Transverse Sections.—Select from lobelia herb (preferably that which has not been compressed) pieces of stem of medium thickness and still bearing the hairy epidermis. Cut several pieces about $\frac{1}{2}$ to 1 inch long. Should they be too hard for section-cutting, soften them by soaking for a few hours in water.

Embed one of these pieces in pith, as described for ergot. Cut several transverse sections, taking care that in one portion at least the whole of the tissue from the epidermis to the hollow centre is included ; it is not necessary that the section should extend over the entire transverse surface.

Place the sections in alcohol ; transfer after a few minutes to water ; mount one in glycerin.

Examination.—Observe the ring of wood enclosing the remains of the pith, the stems being usually hollow. The structure of the wood may be determined by treating it as directed for quassia (or a modification of the method) ; but this study should for the present be deferred and the attention concentrated on the tissues that surround the ring of wood.

Take a fresh section, transfer it to a drop of water on a slide, and spread it out with the needles ; remove the excess of water with filter paper, and drop a drop of chlorzinciodine on to the centre of the section ; cover with a coverslip.

Examine with a low power ; the wood has stained yellow (lignin reaction), while the major part of the tissue exterior to the wood is coloured violet (cellulose reaction).

Examine this tissue with the high power. Observe about midway between the epidermis and the wood a single row of

cells that differ in appearance from the parenchyma exterior to it; the cells are more oblong, tangentially elongated, often flattened, and all the walls, or part at least of the radial walls, are stained yellow. This layer of cells is the endodermis, and this with the tissue exterior to it up to the epidermis constitutes the cortex. The tissue between the endodermis and the wood is composed of pericycle, bast-ring, and cambium.

Examine the epidermis more closely.

The cells of which it is composed are quadratic or nearly so (in transverse section). Occasionally hairs may be found

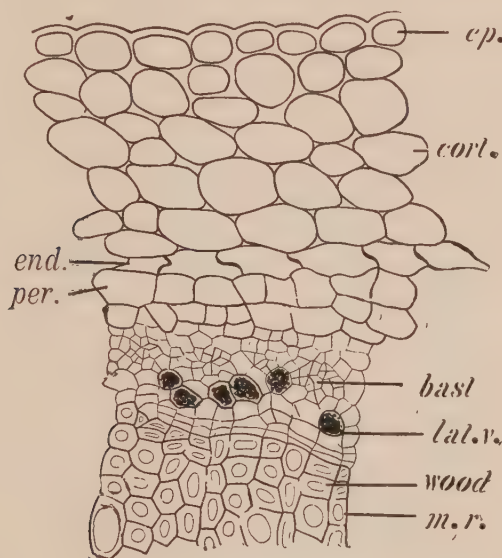


FIG. 49.—*Lobelia inflata*, Transverse Section of Stem. $\times 200$. *cort.*, cortex; *end.*, endodermis; *ep.*, epidermis; *lat. v.*, laticiferous vessel; *m. r.*, medullary ray; *per.*, pericycle.

arising from it; they are one-celled and bluntly pointed; their walls are not very thick.

Next to the epidermis there follow about six rows of parenchymatous cells; these constitute the cortex; the cells are tangentially elongated, and have large intercellular spaces.

The innermost layer of the cortex is the endodermis, the cells of which are closely attached to one another and show no intercellular spaces.

Within the endodermis and abutting immediately upon it is a single or sometimes double row of parenchymatous cells.

These are easily distinguished from the cells of the endodermis, which are thin-walled, flattened, and lignified, as well as from those of the bast, which are much smaller. They constitute the pericycle. In it there may occasionally be found a cell with a thick lignified wall (pericyclic fibre).

Between the pericycle and the wood is the bast (both primary and secondary), but its cells are so small that it is not well adapted for general study.

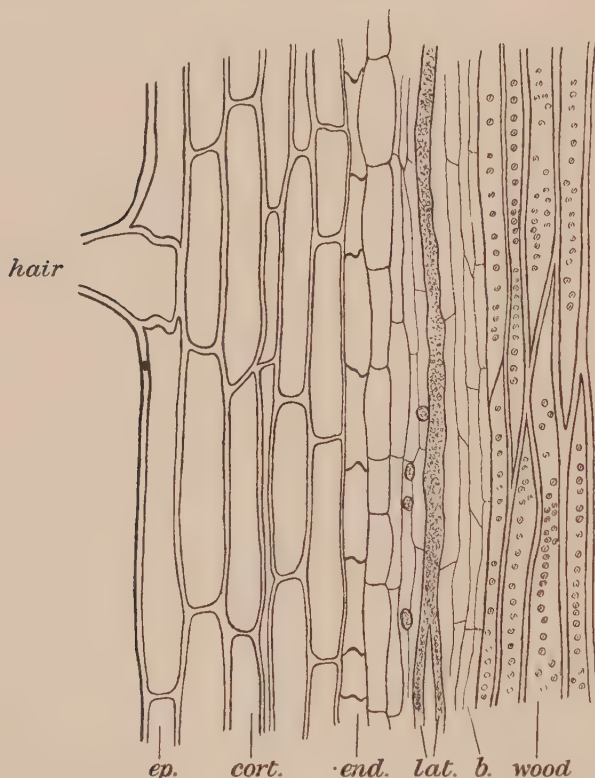


FIG. 50.—*Lobelia inflata*, Radial Section of Stem. $\times 200$. lat., laticiferous vessel; b, bast and cambium.

Observe in the bast scattered cells that are conspicuous by reason of their large size and granular contents; the latter have stained deep yellow with the chlorzinciodine. These are evidently special cells containing a particular secretion, and their nature must be ascertained by further investigation.

Preparation and Examination of Radial Sections.—From the lobelia stem cut a small piece not more than $\frac{1}{4}$ of an inch

long. Split it longitudinally, and embed one half in pith so that the long axis of the lobelia is horizontal and the exposed longitudinal (radial) surface level with the surface of the pith. Hold the pith so that the epidermis of the lobelia is directed outwards. Cut now radial sections, taking care that the razor edge cuts the stem obliquely, passing from the right to the left of the stem as the razor is drawn from heel to point.

Treat the sections as directed for transverse sections ; mount one in chlorzinciodine.

First identify the endodermis by the rectangular flattened shape of the cells and by their lignified walls. Between the

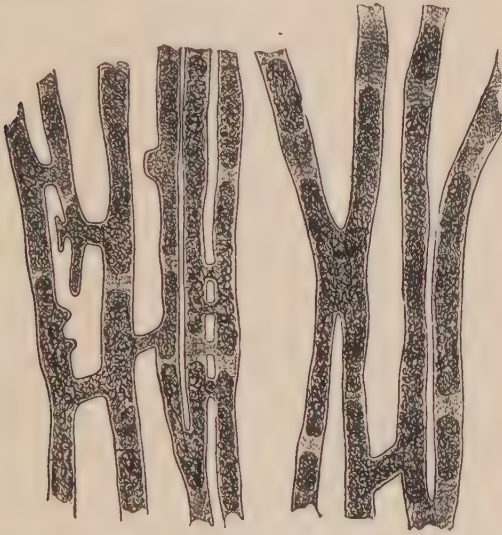


FIG. 51.—*Lobelia inflata*. Laticiferous vessels isolated by potash. $\times 300$.

endodermis and the wood observe and examine the elements with granular contents previously alluded to. They are long tubes, which in favourable sections may be seen to anastomose with one another.

These elements must be examined still more closely, and this can best be done by isolating them from the surrounding tissues. For this purpose digestion with solution of potash may be employed.

Isolation of Laticiferous Vessels.—Take several pieces of lobelia stem about half an inch long ; place them in a test-tube

half full of solution of potash containing about 1.5 or 2 per cent. of caustic potash. Keep them in a water-bath for about half to one hour. Test one from time to time to ascertain if the cortex can easily be stripped off and teased out. When this is the case, pour off the alkaline solution and wash with distilled water. Transfer a piece to a slide, and with the needles strip from it the whole of the tissue exterior to the wood, taking special care to remove everything down to the wood. Wash this tissue with water to remove adhering alkali, and mount in iodine water.

Examine under a low power. Search among the parenchymatous cells for these peculiar tubes, and identify them by their contents, which are now stained yellow. They freely branch and anastomose with one another; they are laticiferous vessels.

Laticiferous vessels have been found in only a few natural orders, and their presence is therefore highly important as a distinctive feature. The following are the principal orders in which they occur: Papaveraceæ, Olacineæ, Papayaceæ, Compositæ (Cichoriaceæ), Campanulaceæ (including Lobeliaceæ), Convolvulaceæ (Dichondra), Euphorbiaceæ (Hevea, Manihot).¹

The structure of the wood can, if necessary, be ascertained by treating it as quassia wood was treated. The complete examination of the cortex is best deferred until the student has examined several leaves and made himself acquainted with the methods there given in detail.

Dulcamara Stem

Source.—The stem, about two or three years old, of *Solanum Dulcamara*, Linn.

Preparation and Examination of Sections.—Select a few medium-sized pieces of dulcamara stem, and soak them for a few hours in water, cut transverse sections, transfer them to spirit, and, after a few minutes, from spirit to water.

Mount one in solution of chloral hydrate, spreading out the section, which is liable to curl, with the needles.

Observe the yellowish ring of wood enclosing the remains

¹ For a complete list see Solereder, *Anatomie der Dicotyledonen*.

of the pith and surrounded successively by bast ring, cortex, and cork. Examine the latter carefully.

The cells of which the cork consists are yellowish in colour ; they have thin wavy walls that are strongly refractive, and exhibit no intercellular spaces ; they contrast sharply with the parenchymatous tissue that follows. They are cork cells. Note their arrangement in regular radial rows, evidently the result of repeated division of the cork cambium cells by tangential walls. The outermost layer bears an intact cuticle, and here and there emergencies or the remains of hairs that have broken off ; it is therefore evidently the epidermis, which has not been thrown off. In this plant the epidermal cell itself divides by a tangential wall ; the outer half is persistent ; the inner half becomes the phellogen, and forms several successive rows of cork cells externally, and subsequently one or two rows of phelloderm cells internally. All these cells are arranged in regular radial rows, a result of their origin. The cork cells can be distinguished by their strongly refractive walls, which also yield the reactions characteristic of suberised membranes (see below). The cells of the phellogen and phelloderm respond more or less distinctly to the tests for cellulose (bluish violet with chlorzinciodine). Those of the phellogen are, naturally, situated between the phelloderm and the cork, and hence may be distinguished by their position. The phelloderm cells have also walls that are thicker than those of the phellogen. The cortex (primary cortex) may be distinguished from the phelloderm by the fact that its cells do not exhibit the regular arrangement that characterises the phelloderm. If, however, much phelloderm is produced, then its cells commonly lose their radial arrangement and become undistinguishable from the cells of the cortex.

The endodermis cannot be easily identified, but its position can be judged. Observe some very thick-walled cells with the cavity almost obliterated ; they are isolated or arranged in small tangential groups, forming an interrupted ring between cortex and bast. These are sclerenchymatous pericyclic fibres, and the endodermis will be the line of cells immediately exterior to them. In some pieces of stem the endodermis contains a notable quantity of starch, which aids in distinguishing it, but this is not always the case.

Within the ring of fibres is the narrow bast ring, recognis-

able by the small irregular cells of the bast, many of which appear filled with dark dust; these will be examined presently. Within the bast ring is the wood, the study of which may be passed over, and within the wood is the remains of the pith, the cells of which are mostly large, rounded, and thin-walled, with intercellular spaces.

Examine carefully the ring of pith, especially near the wood; observe large and small groups of cells, the individual

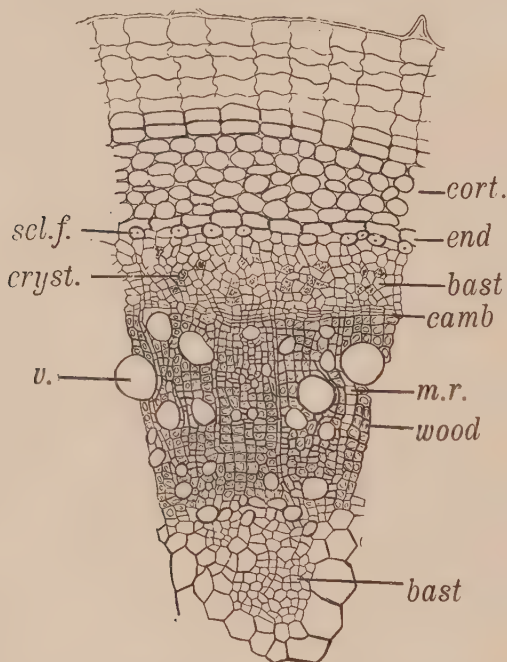


FIG. 52.—*Dulcamara* Stem. Transverse section, showing intraxylary (perimedullary) bast. $\times 80$.

cells of which are easily recognised by their small size; they resemble the cells of the bast. Sometimes an isolated fibre may be found near such a group, and occasionally a cambium has formed. These groups consist of bast tissue, and are intraxylary or perimedullary bast.

Stain a fresh section with phloroglucin and hydrochloric acid. The wood and the cork stain red, and are therefore lignified; the dark dust previously alluded to disappears.

Mount a section in water, focus under the low power, and

irrigate with strong sulphuric acid. The parenchymatous cells of the cortex swell and finally disappear; the wood does the same, but more slowly; the cork cells are not affected. Warm gently until the section begins to turn brown; the wood is further destroyed, the cork is still but slightly affected.

Mount a section in water, add a drop of a solution of 10 grammes of chromic acid in 10 c.c. of dilute sulphuric acid. The parenchymatous cells swell and dissolve with evolution of gas bubbles; the wood behaves similarly, but more slowly; the cork is much more slowly affected.

These two reactions are characteristic of the suberised (cuticularised) cell wall. Of the two that with sulphuric acid is the most generally useful. Another reaction for the suberised wall consists in warming the section gently with strong (20 per cent.) solution of caustic potash; under these conditions suberised cell walls are disintegrated, and small yellowish oily globules make their appearance. Suberised cell walls are also stained red, but not very deeply, when warmed with solution of Soudan red (see list of reagents).

Isolation of Cells containing Sandy Crystals.—The cells containing the dark granular substance resembling dust must next be more closely examined.

Digest a few pieces of stem in a 2 per cent. solution of caustic potash in a water-bath for about half an hour; wash with distilled water; strip off the cortex and bast, and tease out as directed for lobelia. The cells with sandy crystals are seen now to be mostly long and narrow, and under a high power their contents can be resolved into minute (? monoclinic) crystals (see fig. 48); it is sandy calcium oxalate.

Focus a portion of this preparation, or of a radial section, where there are several such cells close together; irrigate with concentrated sulphuric acid very slowly, so as not to create much current. The sandy crystals dissolve and needles take their place; these may remain isolated, or they may form radiating groups. The calcium oxalate is converted into calcium sulphate, which easily crystallises in acicular crystals.

Further proof of the identity of these crystals may be adduced by irrigating a section with acetic acid, in which they are insoluble, and with dilute hydrochloric acid, in which they dissolve without the evolution of gas (which would occur if the substance were a carbonate).

Euphorbia pilulifera

Source.—*Euphorbia pilulifera*, Linn.

Preparation and Examination of Transverse Sections.—

Cut a transverse section of the stem of *Euphorbia pilulifera* as directed for lobelia, and mount in glycerin.

The cells of the epidermis are small in transverse section. Those of the cortical parenchyma (about six or eight rows) are larger with small intercellular spaces, and exhibit tangential elongation.

The endodermis cannot be distinguished either by chlorzinciodine or by phloroglucin and hydrochloric acid, or by any difference in the appearance of the cells or their contents.

Rather nearer to the wood than to the epidermis observe groups of cells that differ from the cells of the cortex in appearance and are arranged in a diffuse circle; they vary in size in different specimens; they are often compressed into an oval, triangular, or polygonal outline; sometimes they exhibit a large lumen. The walls are usually distinctly striated. They are pericyclic fibres, and the layer of cells immediately exterior to these fibres is probably the endodermis, although its cells exhibit no characteristic features.

Between the pericyclic fibres (which are often, but less correctly, termed 'bast fibres' or 'primary bast fibres') and the wood is a narrow ring of bast, the cells of which are often much compressed, and hence indistinct. In this ring of bast observe some few scattered cells which, in transverse section, appear much larger than the other cells of the bast. Direct attention particularly to these cells.

Stain a section with chlorzinciodine. The pericyclic fibres assume first a pink, then red, finally deep red, brown, or black colour. This might be taken to indicate strong lignification, but a section stained with phloroglucin shows them to be but slightly lignified.¹

The large cells in the bast are seen in some cases at least to contain a granular substance that has coloured yellow.

Isolation of Laticiferous Cells.—Digest one or two fragments of the stem with caustic potash, as directed for lobelia. Tease the tissue of the bast with the needles, and examine

¹ The nature of the walls of these fibres requires further investigation.

in iodine water. The cells with large cavities can now be isolated and identified by their brown granular contents. They are long branching tubes, which show no anastomoses as the similar tubes from lobelia stem did; they are laticiferous cells.

Distinguish carefully between laticiferous *cells* and laticiferous *vessels*; the former branch freely, but do not anastomose, the latter exhibit numerous lateral branches which anastomose with neighbouring vessels.

The following are the principal natural orders in which laticiferous cells have been found: Apocynaceæ, Asclepiadeæ, Euphorbiaceæ, Urticaceæ.

Broom Stem

Source.—The young stem of *Cytisus Scoparius*, Link.

Preparation and Examination of Sections.—Cut transverse sections; immerse in spirit, transfer to water; mount one in glycerin. Examine the epidermal cells; they are square or oblong in outline, and possess a thick cuticle. Pass on to the cortex, which is composed of several layers of parenchymatous cells. At intervals the cortex is extended into projections (wings); in each of these wings observe two groups of fibres, one near the epidermis, the other nearer the wood. The fibres are much thickened, the cavity being almost obliterated; the walls show distinct stratification, but are only slightly lignified (test by phloroglucin and hydrochloric acid).

Between the bundle of fibres in the apex of the wing and the epidermis are one or two rows of parenchymatous cells with thickened sides and angles (collenchymatous).

The endodermis cannot be distinguished by appearance from the other cells of the cortex, but may be identified by its position. Observe the interrupted band of fibres several rows wide near the wood; these are developed from the pericycle, and the row of cells immediately exterior to them is the endodermis.

Examine these fibres; the outline of each is oval or polygonal; the wall is thick and shows stratification; the cavity is reduced to a slit.

Within the ring of fibres is a narrow ring of bast followed by wood. Neither bast nor wood need now be minutely examined.

The pith consists mostly of very large thin-walled cells.

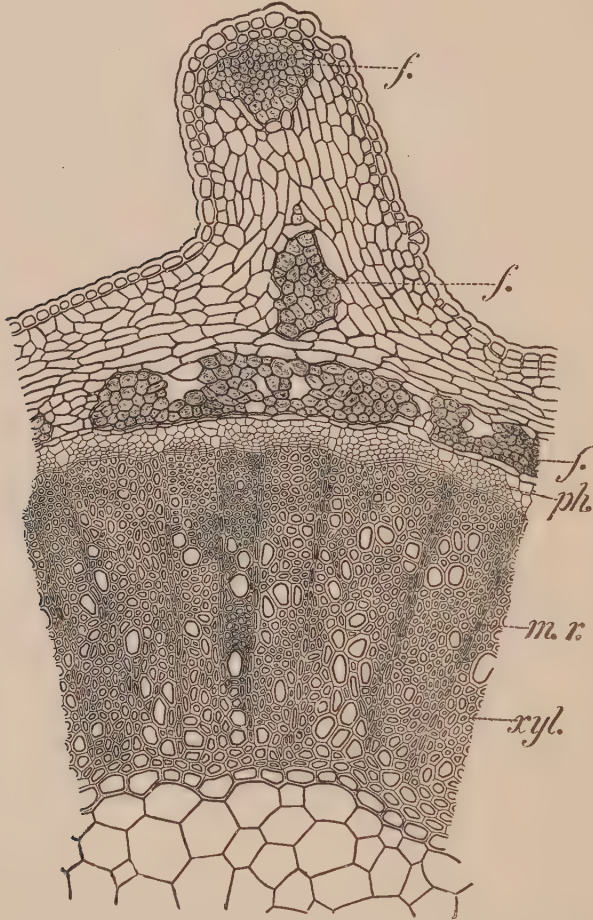


FIG. 53.—Broom, transverse section of young stem. *f.*, fibres; *m.r.*, medullary ray; *ph.*, phloem; *xyl.*, xylem. $\times 100$.

SECTION VII

LEAVES

INTRODUCTION

ALTHOUGH leaves differ in structure and organisation from the axes upon which they are borne, yet their tissues are usually continuous with and analogous to, or even identical with, those of the stems that produce them. The epidermis of the stem is continuous with that of the petiole and lamina of the leaf; the cortex of the stem passes into the cortex of the midrib, of which the mesophyll is but a specially organised development. A portion of the stele leaves the stem and passes into the leaf, where it constitutes the meristele of the midrib and eventually subdivides into the schizosteles of the veinlets.

In discussing the general anatomy of leaves it is advantageous to deal first with the epidermis and its appendages, then with the mesophyll, and lastly with the veins.

Epidermis.—The epidermis usually consists of a single layer of cells, the shape of which varies in the leaves of different species but is constant in all plants of the same species growing under normal conditions.

In transverse section the epidermal cells usually exhibit a rectangular outline, this being especially the case with those from the interneural spaces, the cells lying above or below the midrib being rounded at the angles and therefore more or less oval.

The cuticle varies in thickness, being generally thick in shrubs (jaborandi) but thin in herbaceous plants (hemlock). It is commonly smooth, but sometimes exhibits projecting ridges (belladonna) or protuberances (coca); in surface view the former appear as striations, the latter as ill-defined circles.

The epidermal cells may appear, in surface view, either comparatively small and straight-walled (buchu, coca, jaborandi), or large, with thin, undulating, or even strongly wavy walls (foxglove, hemlock, henbane, belladonna). In powdered herbs the epidermal cells usually present their surface, not their section, to the observer, and the study of the differences thus exhibited becomes therefore extremely important.

Stomata.—Most leaves bear stomata, either upon the upper or lower surface or both, but submerged leaves are usually free from them. When they occur they are not necessarily uniformly distributed over the surface that bears them, although this is the more frequent occurrence, but are sometimes grouped in various ways (bearberry). Very often the cells surrounding the guard-cells of the stomata assume a particular arrangement, which is constant for the same species and often recurs in a number of species belonging to the same natural order. Thus in the coca leaf the stoma is surrounded by four cells, two of which have their long axes parallel to the ostiole; in senna a similar arrangement is met with. In labiate leaves, on the other hand, the stoma is enclosed between two cells the long axes of which are at right angles to the ostiole. In jaborandi there are several narrow cells tangentially arranged round the stoma, while in leaves derived from Solanaceæ and Compositæ there are usually three or four cells, one of which is smaller than the others. Nor are the stomata always inserted at the same level, for while some are elevated above the epidermis, others may be sunk below it, the latter being especially the case with plants growing in arid districts (senna).

Hairs.—Many leaves, including several official ones, are glabrous (coca), but it is more common to find hairs either distributed over the lamina or restricted to the veins. These hairs exhibit an infinite variety in shape and nature, but they are constant in the same species, and even different species of the same genus are often found to be furnished with similar hairs. They possess, therefore, very great diagnostic value. Hairs may be divided into two classes—viz.: simple (or protective) hairs and glandular (or secreting) hairs. These two classes may be considered separately.

- (a) *Simple Hairs.*—These may be either unicellular or pluricellular, according as they consist of a single cell or of several cells. If the cells are arranged in a single row,

the hair is uniserial; if in several rows, pluriserial. The length and shape of the hair, the thickness of the wall, and the nature of the surface should be carefully studied; thus in senna the hairs are one-celled, thick-walled, and warty, in foxglove they are uniserial, pluricellular, and the walls are thin and slightly warty, and so on.

- (b) *Glandular Hairs*.—These are distinguished by their terminating in a cell or collection of cells that secrete resin, volatile oil, &c. The secreting cells are, therefore, borne upon pedicels, which, like simple hairs, may be unicellular or pluricellular, uniserial or pluriserial. Very often the pedicel is unicellular and short.

The secreting cell, or collection of cells, is termed a gland. Sometimes it remains unicellular, but often it divides by vertical walls or horizontal walls, or both, and thus becomes pluricellular and pluriserial (*Solanaceæ*, *Compositæ*). When division takes place by vertical walls only, it does so in a very regular manner, dividing the gland into two, four, or eight similar cells (bicellular, quadricellular, octocellular glands).

Unicellular or bicellular glands are commonly rounded or oval; they are inserted on the epidermal cells, and when they fall off leave circular scars either near the centre or close to one of the lateral walls.

Quadricellular and octocellular glands are larger and often situated in depressions on the surface of the leaf; they are usually borne upon very short pedicels, which are inserted between several epidermal cells, and consequently they leave, when they fall off, a scar differing from that left by a unicellular or bicellular gland.

All these hairs are best seen in surface preparations.

Mesophyll.—The mesophyll is said to be *homogeneous* when the cells of which it consists exhibit but little difference in shape, &c. (savin leaves), *heterogeneous* when they assume distinctly different forms. The latter is by far the more common, most leaves exhibiting a palisade tissue well differentiated from the spongy parenchyma. Two forms of heterogeneous mesophyll are known—viz. *dorsiventral* and *isobilateral*. Most leaves possess a distinctly dorsiventral mesophyll, and the presence of an isobilateral structure should, therefore, be

carefully noted. The shape and size of both the palisade and the spongy parenchyma cells should be carefully observed, as well as the nature of their contents. Among the latter the presence or absence of crystals, and, in the former case, their shape and nature, afford valuable evidence in establishing the identity of a leaf. They generally consist of calcium oxalate, and are usually rather small. Whenever they exist in a leaf they are always to be found in the powder, however fine it may be. Some leaves (foxglove) are entirely free from crystals, but most officinal leaves contain them distributed throughout the mesophyll, the cortex of the midrib, and the bast of the midrib and veins. Their shape generally remains constant in leaves of the same plant, but it may vary for different plants of the same natural order. Thus, in belladonna leaves the calcium oxalate usually assumes the form of sandy crystals, in henbane that of small prisms, in stramonium that of cluster crystals. In senna leaves both prisms and cluster crystals are found.

The mesophyll may also contain various forms of secretory tissue, such as oil cells, oil glands, secretory ducts, laticiferous cells, laticiferous vessels, &c. The following are the principal natural orders in which these secretory cells &c. occur:¹

Secretion Cells.—Calycanthaceæ, Magnoliaceæ, Anonaceæ, Nymphæaceæ, Canellaceæ, Rutaceæ, Simarubeæ, Meliaceæ, Compositæ (some), Sapotaceæ, Piperaceæ, Chloranthaceæ, Myristiceæ, Monimiaceæ, Laurineæ.

Elongated Secreting Tubes, with brown contents.—Anacardiaceæ, Leguminosæ (Papilionaceæ, Mimoseæ), Compositæ, Myristiceæ, Monimiaceæ, Euphorbiaceæ.

Internal Schizogenous Glands.—Hypericineæ, Guttiferæ, Rutaceæ, Myrtaceæ, Rubiaceæ, Compositæ.

Secretion Ducts.—Hypericineæ, Pittosporæ, Guttiferæ, Dipterocarpeæ, Burseraceæ, Anacardiaceæ, Leguminosæ (Cæsalpinieæ), Umbelliferæ, Araliaceæ, Compositæ (Cichoriaceæ).

Laticiferous Cells.—Apocynaceæ, Asclepiadeæ, Euphorbiaceæ, Urticaceæ (Humulus and Moreæ).

Sclerenchymatous cells or fibres are also occasionally present, (tea, witchhazel), and then constitute important diagnostic characters (compare 'Tea').

Midrib.—The elements of which the midrib and lateral veins consist may sometimes be turned to account in identifying

¹ For complete list see Solereder, *Anatomie der Dicotyledonen*.

a leaf, although their structure presents general features common to most leaves.

Next to the epidermis there is usually to be found a layer of collenchymatous cells of varying extent passing into the normal cortical parenchyma (or ground tissue) of the midrib. In the powdered leaves the collenchymatous cells usually exhibit their length, and are easily distinguished by their elongated shape and thickened walls.

The cells of the cortical parenchyma, on the other hand, in surface view have thin walls, and are rounded or polygonal in herbaceous plants, but usually rectangular or elongated in the leaves of shrubs.

The wood of the midrib and lateral veins may contain vessels, tracheids, &c., the size of which is sometimes important; the bast seldom offers valuable indications, but the presence or absence of well-developed pericyclic fibres, their shape, the extent of the thickening and lignification should be ascertained, as these characters are often very important.

General Scheme of Examination

In the complete and thorough examination of a leaf the following course of procedure may be with advantage adopted:

- (1) The preparation and examination of transverse sections, including the midrib, the interneural regions, and the lateral veins;
- (2) The preparation and examination of longitudinal sections of the midrib;
- (3) The isolation and examination of pericyclic fibres, if such are present;
- (4) The separation and examination of the epidermis of both surfaces;
- (5) The preparation and examination of the powder.

In examining leaves the student should remember that an accurate knowledge of their structure should bear the following practical results: (1) the identification of the leaf in its entire state or at least in coarse fragments; (2) the identification of the powdered leaf. The first of these objects is generally accomplished by the examination of transverse sections and of

surface preparations of the epidermises. But before examining a powdered drug it is necessary to be accurately acquainted with the structure of that drug, with the size, shape, and other characters of the various histological elements of which it is composed, for otherwise it would be impossible to say whether particular elements observed in the powder are derived from the drug in question or from some foreign source constituting in the latter case an impurity or an adulteration. Moreover, it occasionally happens that certain characters that are conspicuous in the entire drug become obliterated during the process of pulverisation. Thus large oil glands which form a conspicuous character in some drugs are so destroyed by pulverisation as to be with difficulty found in the powder. On the other hand, it is not uncommon to find certain layers of cells tenaciously retaining their relative position in the powder and presenting certain characteristic aspects.

The examination of the powdered drug is further a useful supplement to the examination of the entire drug, as details may thereby be brought to light that would otherwise escape observation.

Examination of Powdered Drugs

The following media will be found generally useful in mounting powders for microscopical examination. They must, however, be supplemented by special methods of examination adapted for particular drugs; these methods will be described in detail as the student progresses to the study of the drugs for which they are required.

Water.—One of the principal advantages of water as a medium in which to examine powdered drugs is that it exerts very little prejudicial influence upon the cell walls or cell contents. The latter, so far as they are insoluble in water, remain unaffected, and in this respect water is much less objectionable than chloral hydrate or caustic potash. Delicate details are also more easily visible in water than in either chloral hydrate or glycerin. On the other hand, water is destitute of that power of rendering tissues transparent that is possessed both by chloral hydrate and glycerin, and hence underlying tissues that are visible in either of these media may be entirely hidden from view in water. The solvent power of chloral hydrate on many cell

contents (remains of protoplasm, starch, chlorophyll, and many other substances) to which its clearing action is partly due renders it in so far inferior to water as it exhibits the cell contents after they have been more or less altered by the action of the mounting medium.

Previous to examination under the microscope the powder should remain in contact with water for at least a few hours, in order to allow the cells and cell walls to be thoroughly penetrated.

Glycerin, pure or diluted.—Glycerin is useful, as it renders tissues more transparent than water does. It has a much less injurious influence upon the cell walls and cell contents than chloral hydrate, and, especially if used diluted, is very useful. Unlike water and chloral hydrate, it is of course not liable to evaporate or crystallise, and therefore preparations in glycerin may be kept for a considerable time under observation. The examination both in dilute glycerin and in water should not be omitted, as it frequently reveals valuable details. A good method of procedure is to mix the powder thoroughly with dilute glycerin and allow several hours for the liquid to penetrate the tissues before examining them.

Solution of Chloral Hydrate.—This induces a swelling of dried and contracted cell walls which makes the cells assume their normal size and shape, a function which may, however, be disadvantageous, as very delicate cell walls may become abnormally swollen. At the same time its powerful solvent action on starch, proteid matter, resin, and other substances makes it an excellent clearing agent, and its high refractive power renders the particles more transparent than either water or glycerin. Naturally these two functions may also be disadvantageous, because important cell contents may be removed and delicate markings may be rendered invisible. But as a general mountant for powders for the exhibition of the more resistant cell walls, for expanding the cells, and rendering thicker particles more transparent, it is extremely useful.

Solution of Potash.—This is a more powerful reagent than solution of chloral hydrate. It induces, especially if concentrated (20 to 50 per cent.), a very vigorous expansion of the cell wall, and will therefore cause tissues to resume their normal shape when solution of chloral hydrate fails. It

dissolves starch and many cell contents. It is especially useful for resistant tissues and for thick, leathery leaves, particularly if they contain much colouring matter derived from tannin, but it should not be forgotten that it may induce very considerable alteration in the cell wall.

Bearberry Leaves

Source.—The leaves of *Arctostaphylos Uva Ursi*, Linn.

Transverse Sections.—Soak a few bearberry leaves in water for several hours; cut one transversely at a point distant from the base about one-third of the length of the lamina. Sections of the midrib should always be taken at this point, as the structure of a section near the apex is often very different

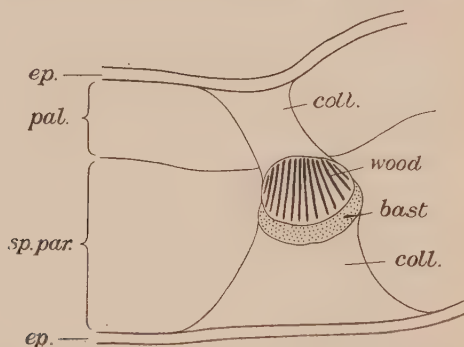


FIG. 54.—Bearberry Leaf. Diagrammatic section of Midrib, showing the distribution of the various tissues. $\times 65$.

from that of one near the base. Fix the lower part of the leaf in pith, and cut transverse sections; transfer them at once to alcohol.

Place one in a drop of water on a slide; remove the water, drop on a drop of glycerin, and cover.

Examine the midrib.

The wood is easily distinguished. It is fan-shaped. The elements are small but distinct; the medullary rays (high power) are radially elongated, and most of them are filled with a brown substance.

Below the wood is a crescent-shaped bast, which extends rather more than halfway round the wood. The cells of this tissue are also very small, but under the high power and in favourable sections they may be distinguished.

The endodermis is not well marked, and cannot with certainty be identified.

Above and below the meristele a mass of cells with much thickened walls and angles (collenchymatous) connect the meristele with the epidermis of both surfaces. Many of these cells (especially those near the midrib) contain prismatic and cluster crystals of calcium oxalate. Their nature can be determined by radial sections, but the student is advised not to attempt this for the present. Immediately above the wood between it and the bridge of thick-walled cells, a few scleren-

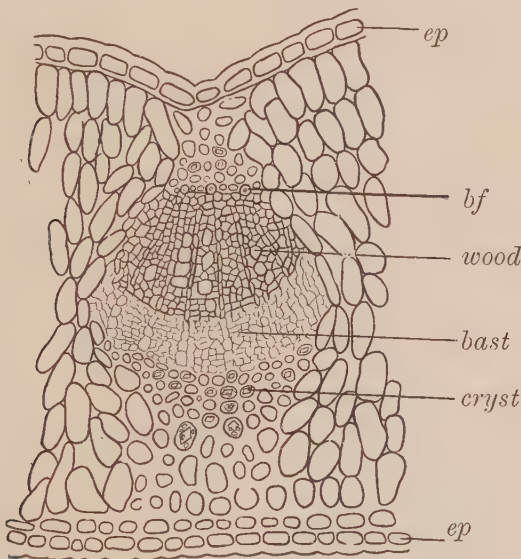


FIG. 55.—Bearberry Leaf, transverse section. $\times 120$.

chymatous fibres with very thick walls and small cavities can usually be seen (fig. 55, *bf*); they are smaller than the thick-walled cells, but larger than the elements of the wood, from which they also differ by their rounded outline and thicker walls.

The cells of the epidermis are remarkable for their very thick cuticle.

Make a diagrammatic sketch of the section (compare fig. 54).

To another section add solution of chloral hydrate, cover, and after a couple of minutes draw off the chloral hydrate and allow glycerin to take its place.

The section clears rapidly in chloral hydrate. The crystals of calcium oxalate can now be seen very distinctly as dark masses filling the cells in which they occur. In the bast the cell walls may have swollen a little.

Examine the interneural mesophyll. It shows on the upper side several (two to four) rows of short palisade cells; here and there an occasional crystal of calcium oxalate may be observed. The lower portion of the mesophyll consists of spongy parenchyma.

Observe the lateral veins. Like the midrib, they are connected with the epidermis by a bridge of thickened cells, and are also accompanied by fibres, which are more numerous in proportion to the wood than they are in the midrib.

Stain a section with chlorzinciodine. Observe the thickness of the cuticle and cuticular layers, which stain yellow.

Surface Sections.—Next proceed to examine the surface of the epidermis. For this purpose surface sections must be prepared.

Take a soaked leaf, bend it over the forefinger and cut thin sections from the upper surface. Only the first section from any particular point can be utilised. Transfer these to alcohol on the slide, taking care to have the cuticular surface uppermost. Replace the alcohol by water and the water by glycerin.

The outlines of the cells can be distinctly seen, especially near the edge of the section. They are polygonal, the walls being often slightly wavy; there are no stomata.

Focus the epidermal cells on a thicker part of the section and then gradually focus down until the palisade cells which lie below the epidermis of the upper surface (compare transverse section) appear distinct. Observe that the palisade cells are rounded in outline, are rather smaller than the epidermal cells, and have thin walls; they are often seen when examining the surface preparations of leaves.

Sketch a few epidermal cells together with a few of the subjacent palisade cells as they appear in a surface section.

Cut and examine in the same way sections from the under surface of a leaf.

The epidermal cells are similar to those of the upper surface, but are generally rather larger, those above the veins showing a little difference from those above the interneural

regions. The latter bear stomata, often apparently arranged in groups. Examine one of the stomata more closely under the high power; it is surrounded by six or eight cells, which, however, show no definite arrangement.

With the low power examine a larger area; hairs or glands are very seldom to be found.¹

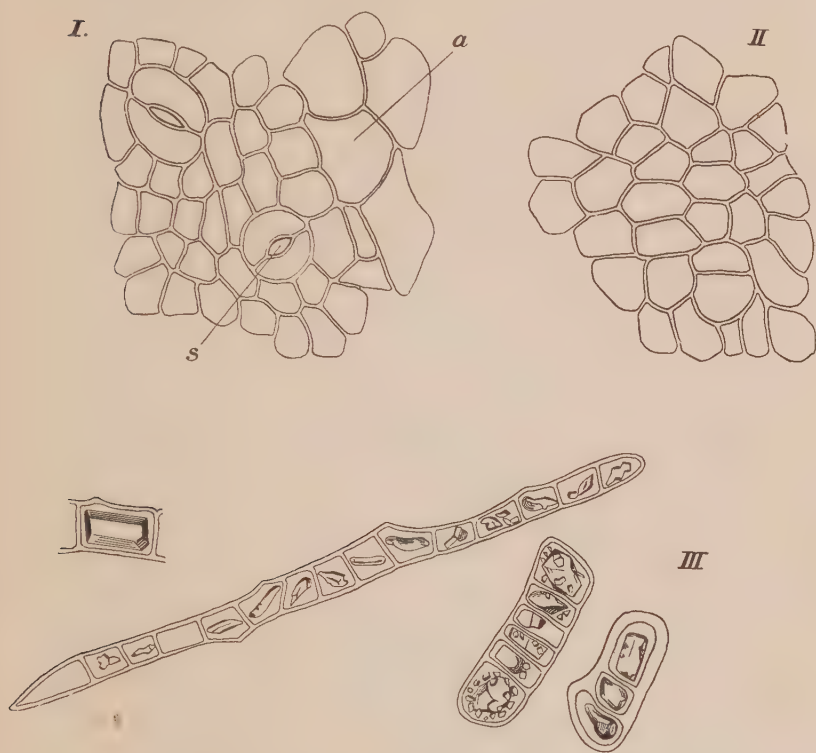


FIG. 56.—Bearberry Leaf. *I.*, lower epidermis; *a*, portion over a vein; *s*, stoma; *II.*, upper epidermis; *III.*, cells from cortex of midrib, showing calcium oxalate; isolated by maceration with potash. $\times 220$.

Separation of the Epidermis by Caustic Potash.—Macerate a few pieces of the leaf for half an hour in 2 per cent. solution of caustic potash; wash several times with distilled water.

Transfer a piece to the slide, and with the needles strip the epidermis from both under and upper surfaces; free it from

¹ In the powder, short, one-celled, thick-walled, conical hairs are occasionally to be found, as well as glandular hairs; these are derived from the margin of very young leaves.

adhering cells by brushing with a camel's-hair brush, and mount in water, taking care that the cuticular surface is uppermost.

The upper epidermis shows an appearance very similar to that observed in the surface section.

In the lower epidermis it will probably be observed that the cell walls, instead of being sharply defined, are indistinct and granular. This is due to the action of the caustic alkali.

Separation of the epidermis by maceration in caustic potash often yields excellent results, especially in cases in which the preparation of surface sections is difficult. The strength of the solution of potash and the length of time during which the heating should be continued vary with the nature of the leaf. Usually thin leaves require a weaker alkaline solution (0.2 to 1 per cent.) and a shorter maceration (10 to 20 minutes) than thick, leathery leaves. The foregoing comparison shows, however, that it is always desirable to ascertain by surface sections or other preparation whether the caustic alkali has materially altered the appearance of the cell walls.

Examination of Crushed Leaves.—The student should now proceed to examine coarse fragments of bearberry leaves, with a view to identifying them as such.

Coarsely crush some bearberry leaves and remove the finer fragments by sifting on a No. 20 sieve. Examine the coarser pieces as follows :

Soak them in water until sufficiently soft for cutting. Select some of the larger pieces, embed them in pith, and cut sections, taking care, if possible, to cut the veins at right angles. If necessary, fix the smaller fragments on to the pith by means of a little gum and glycerin (see list of reagents), which should be allowed to dry sufficiently to hold the leaf fast.

Treat the sections as described above. Examine and sketch the transverse section of a vein and also of an interneural portion. Compare these sketches with the sketches made during the examination of the leaf itself. In the veins note particularly the bridge of thick-walled cells which connects the meristele with both upper and lower epidermis ; also the sclerenchymatous fibres above the wood. In the section of the interneural portion of the lamina note the thick cuticle and the several layers of palisade.

Next digest some of the fragments in solution of potash,

wash, and with the aid of needles strip the epidermis of both surfaces. Examine and sketch these, and compare the sketches with those of the leaf.

Indian Senna Leaves

Source.—The leaflets of *Cassia angustifolia*, Vahl.

Preparation for Cutting.—Select a few large and well-preserved Indian senna leaves; allow them to remain in a moist atmosphere until they are supple, but not longer, or they will become too moist. As a rule, this treatment is the most suitable for thin, papery leaves, such as senna, while soaking is preferable for thick, leathery leaves, such as bearberry. The condition that is most favourable for section cutting has been described in Section IV (Ergot).

Preparation of Transverse Sections.—Cut several leaves transversely through the midrib at the point at which the sections are to be taken (one-third of the distance from base to apex), and cut away the lamina on each side of the midrib until the width is reduced to about one-quarter or one-eighth of an inch. Place four or five such pieces one on the other, keeping the cut edges level, and embed the entire packet in elder-pith so that every stroke of the razor will cut a number of sections at once, instead of one only; this method is advantageous for thin leaves. Transfer the sections, as they are cut, to alcohol.

Mounting and Examination.—Select a thin section, transfer it to a drop of water on a slide; replace the water by dilute glycerin. Examine the midrib. The wood is fan-shaped. Below and on either side of it observe delicate, small-celled, bast tissue, forming nearly a semicircle. Above the wood there are a few rows of thin-walled parenchymatous cells. Both above and below there is a protecting shield of more or less thickened, pericyclic fibres: this shield is crescent-shaped below the wood, but oval above it. Beyond the shield of fibres on the under surface is thin-walled parenchyma, which rapidly passes into collenchyma; on the upper side there is palisade tissue above the meristele.

Make a diagrammatic sketch of the section.

Mount another section in chloral hydrate. The cells expand and the tissue is cleared, proteid matter, &c. dissolving. The

strands of bast become very distinct ; the parenchymatous cells on the outer margins of the arcs of fibres contain prismatic crystals of calcium oxalate.

Examine now the section of the interneural portions of the lamina. The cells of the upper and under epidermis are nearly

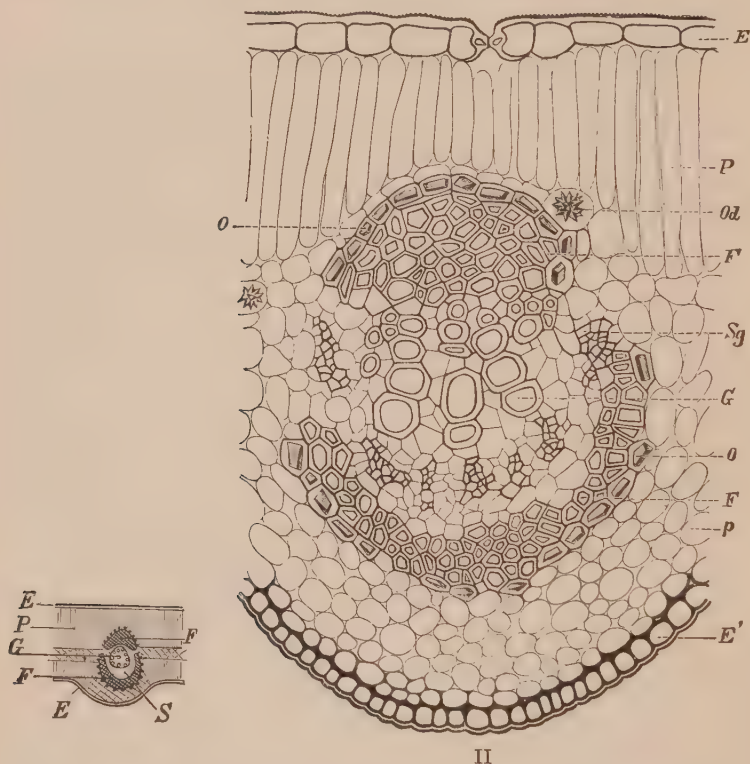


FIG. 57.—Senna Leaf, transverse sections of midrib. I. Diagrammatic; *E*, epidermis; *F*, pericyclic fibres; *G*, wood; *P*, palisade; *S*, bast. II. Magnified 210 diameters; *E*, epidermis of upper, *E'* of lower surface; *F*, pericyclic fibres; *G*, vessels; *O*, prismatic crystals of calcium oxalate; *Od*, rosette crystals of same; *P*, palisade; *p*, cortical parenchyma; *Sg*, sieve tissue. (Meyer.)

square in outline, and covered by a distinct cuticle that bears a fine granular coating (of wax). Some of these epidermal cells appear divided into two cells by tangential walls, which are sometimes straight, but often bulge into the upper part. This appearance has been proved to be due to mucilage, which is deposited on the inner tangential wall of the epidermal cell

and is covered by a thin layer of cellulose ; when immersed in water the mucilage swells, becomes transparent, and produces a bulging of the cellulose covering it.

Take a fresh section from alcohol, allow it to expand for a few seconds in chloral hydrate on the slide, wash quickly with a drop of water, and add a drop of ruthenium red solution ; the mucilage in such of the cells as are intact will stain bright red (the collenchymatous tissue of the midrib and the sclerenchymatous fibres will also be stained).

Continuing the examination of the leaf section, observe the stomata which may be found on both surfaces of the leaf ; the guard-cells are sunk below the level of the epidermis. Occasionally a hair may also be seen, but these are comparatively seldom found in sections. They are one-celled, thick-walled, blunt-pointed, and covered with minute warts ; the base is conical, and it is wedged between two epidermal cells.

Pass now to the interneural mesophyll. Note on both upper and under surfaces a layer of long narrow palisade cells (isobilateral structure), the walls of which are more (under surface) or less (upper surface) wavy. Between the palisade tissues there is a narrow layer of spongy parenchyma the cells of which are mostly rounded ; here and there a cluster-crystal of calcium oxalate can be found. Through the spongy parenchyma lateral veins and veinlets run ; these possess a structure similar to that of the midrib, the elements gradually diminishing in number until the fibres disappear and the wood is reduced to a single vessel. They are often cut obliquely, and then do not exhibit their structure clearly.

Sketch a part (about 3 or 4 cells wide) of the interneural portion of the leaf.

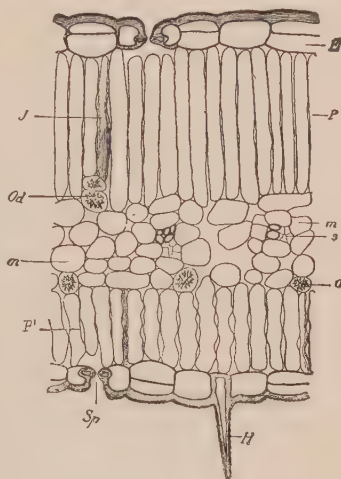


FIG. 58.—Senna Leaf, transverse section of interneural portion of lamina. *E*, epidermis ; *H*, hair ; *J*, palisade cell containing a strongly refractive substance ; *m*, mesophyll ; *P*, palisade ; *Od*, calcium oxalate ; *s*, bast ; *Sp*, stoma. $\times 210$. (Meyer.)

Preparation and Examination of Longitudinal Sections.—

Examine the structure of the midrib more closely. Prepare a leaf for longitudinal section through the midrib by cutting out a piece about one-eighth or one-quarter of an inch wide, and embedding it so that the midrib is just level with the surface of the pith. Cut longitudinal sections, and transfer them to alcohol. Mount in chloral hydrate, and examine under the low power. If there is much air warm gently until this is expelled; as a rule, air is more easily removed from transverse than from radial sections.

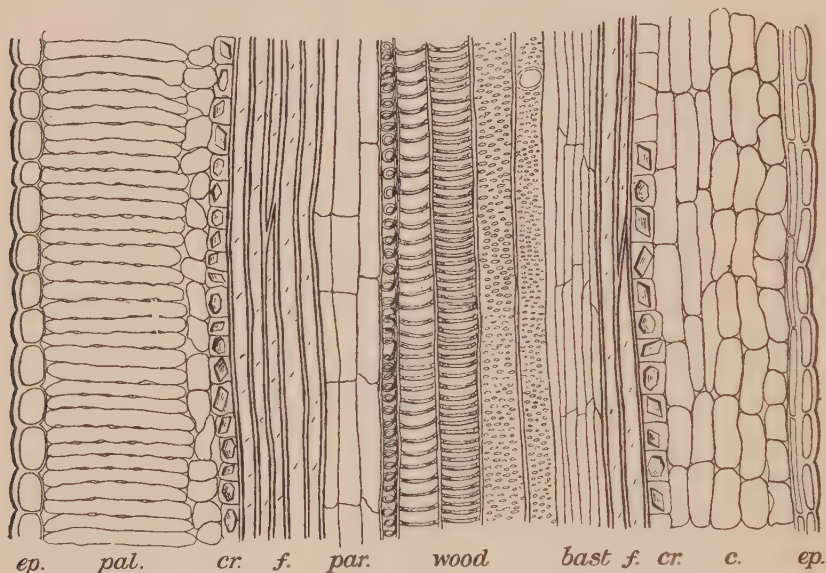


FIG. 59.—Senna Leaf, radial section of midrib. $\times 180$. *c.*, cortex; *cr.*, crystals of calcium oxalate; *ep.*, epidermis; *f.*, pericyclic fibres; *pal.*, palisade; *par.*, parenchymatous cells above the wood.

Select a section that has passed through the centre of the midrib—possibly several leaves will have to be cut before a satisfactory one is found—add a drop of glycerin to prevent the choral hydrate from crystallising. Examine under the high power, and compare the tissues observed with those which the transverse section has shown to be present.

The upper epidermis can be recognised by the layer of palisade that is beneath it, as shown by the transverse section; its cells are slightly axially elongated. The palisade tissue

beneath the epidermis resembles that of the interneural mesophyll, and exhibits the same irregularity in its walls. A row of cells of varying shape, but approximately isodiametric, separate the palisade cells from the crystal cells that abut upon the pericyclic fibres. If the latter are not readily distinguished, they may be made more conspicuous by appropriate staining; they are strongly elongated, rather thick-walled, and exhibit scattered slit pores arranged in a left spiral. The crystal cells are small, slightly axially elongated, and contain a single crystal of calcium oxalate in each.

Next to the pericyclic fibres are several rows of axially elongated parenchymatous cells which abut upon the vessels of the wood. The latter are spiral, annular, and pitted in succession.

The bast which follows the wood is, as a rule, but indistinctly seen. Here and there on a particularly favourable section very long narrow cells may be distinguished.

The bast is bounded by pericyclic fibres similar to those already described; next to the fibres there is a row of crystal cells.

The remaining tissue consists of the cortical parenchyma of the midrib. It is composed of axially elongated cells, which become collenchymatous near the epidermis. The cells of the latter are much smaller and more strongly axially elongated than those of the upper surface.

Further information respecting the elements of which the midrib consists may be obtained by cutting the thicker portions of the midrib from several leaves and digesting them with Schultze's maceration mixture as directed under Quassia Wood. The shape of the pericyclic fibres, vessels, &c. may be studied in this preparation.

Examination of the Epidermis.—It is frequently difficult to separate the epidermis of thin leaves by digestion with solution of potash, and recourse may then be had to the following mode of procedure.

Cut a senna leaf into small pieces about one-eighth or one-fourth of an inch square; place two side by side on a slide, the one with the upper, the other with the under surface uppermost. Add several drops of solution of chloral hydrate, cover with a coverslip, and gently warm until the chloral hydrate just begins to boil; keep it near the boiling-point for a few moments, then cool, replace the evaporated liquid by more

solution of choral, and examine. If the operation has been successful the leaf will have been rendered so transparent that the epidermal cells, stomata, and hairs will be distinctly visible, but some portions of the epidermis will exhibit the cells better than others, and some leaves respond better to this treatment than others.

Observe that the epidermal cells are thin-walled and polygonal in outline; stomata are found on both sides; they are usually placed between two cells, to the longer axes of which the ostiole is parallel; the latter is depressed beneath the level of the epidermis (since the microscope tube must be depressed to focus it). The hairs are very conspicuous; they are one-celled, thick-walled, and warty, and are often curved. Observe also small rounded scars which are left when the hairs fall off.

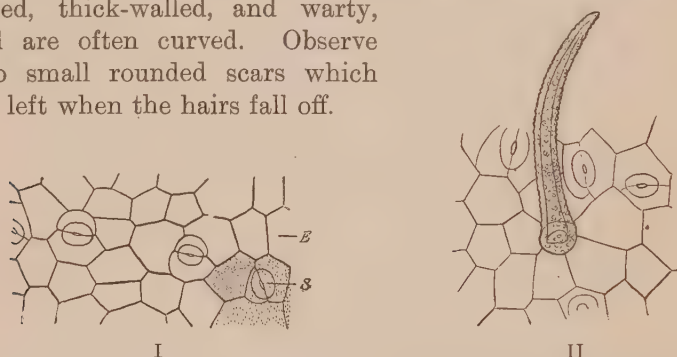


FIG. 60.—Senna Leaf. I. Epidermis of upper surface. II. Epidermis of lower surface. *E*, epidermal cell; *S*, stoma. $\times 210$. (Meyer.)

The distribution of the hairs, stomata, &c., can be well studied in fragments of the leaf made transparent in the manner described.

Depress the tube of the microscope until the palisade tissue is focussed; observe that the cells appear as small circles. Focus still farther down, below the palisade tissue, until the midrib (or a lateral vein) is focussed, and observe the regular rows of crystals; these are the calcium oxalate crystals that were seen in transverse section near the arc of fibres. They are now seen to be arranged in long axial rows.

Comparing the epidermis of the upper surface with that of the lower, observe that they are very similar; the epidermal cells have the same shape, stomata are present, and palisade tissue follows the epidermis in both cases. The lower surface, however, usually bears more hairs than the upper. Most leaves

exhibit differences sufficiently marked to enable one to distinguish the epidermis of the upper from that of the lower surface; thus stomata are often absent from the upper surface, and palisade tissue is not, as a rule, to be found abutting on the lower epidermis.

Sketching.—It is very necessary that the student should accustom himself to recording by suitable sketches the details of the tissues observed in order that later on he may compare such details with those observed in the powdered drug. As the fragments of most powdered leaves seldom show transverse sections of the interneural mesophyll, and still more seldom transverse sections of the midrib, diagrammatic sketches of these should for the present suffice (compare figs. 54, 57, I.). The *details* of the various tissues and elements should, however, be carefully drawn, care being taken to avoid all unnecessary multiplication of similar cells and to retain the correct relative size. Fig. 61 shows the structural details of a senna leaf and the manner in which they should be recorded for future reference.

Examination of Powdered Senna.—The student will now have advanced so far as to be in a position to undertake the examination of a powdered drug, and for this purpose he may select powdered senna.

It will be very undesirable for him, for the present at least, to examine any powdered drug until he has made himself acquainted with the anatomy of that drug by sections and other treatment, as directed in the preceding chapters. Indeed, in any case the most rational course to adopt is first to determine the macroscopical characters of the drug, next the minute structure as revealed by careful, systematic investigation, and, lastly, to ascertain the changes that these tissues undergo when subjected to the operation of powdering, and to identify them in that state.

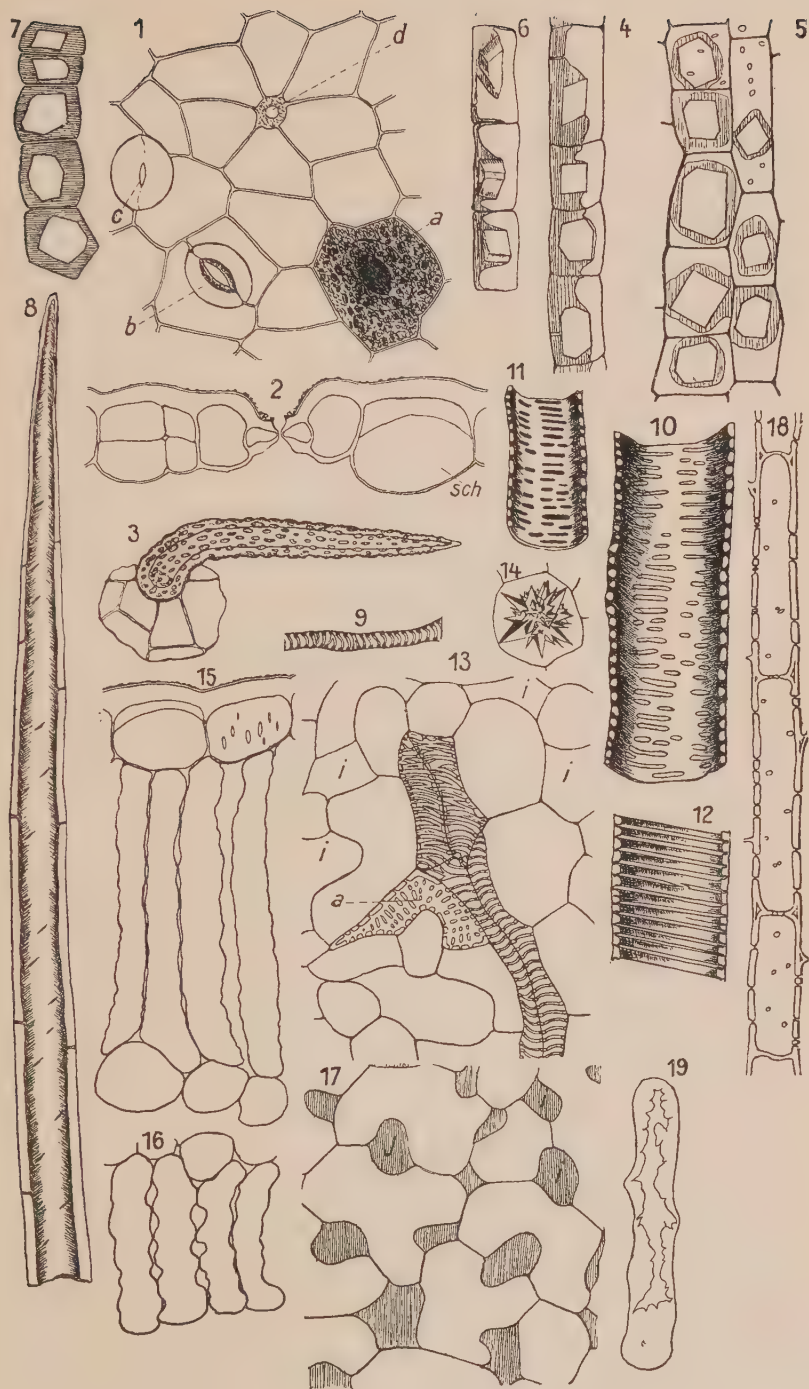
A little consideration will show that the effect of pulverisation will probably be greater upon soft and delicate tissues than upon tough and hard ones, and that it will be more marked as the extent to which the pulverisation is carried increases. Sclerenchymatous fibres with comparatively thick walls may therefore be expected to resist pulverisation better than vessels, the walls of which are comparatively thin, and these again better than parenchymatous cells, the walls of which are not lignified. Moreover, probably those cells which are firmly

held together in the drug will remain more or less firmly attached to one another when the drug is powdered. Such collections of cells may also present themselves to observation in positions different from those that they occupy in carefully prepared sections. Oblique views of various groups of cells will probably be met with, and be more difficult of interpretation than accurate sections, not only on account of their obliquity, but also on account of the thickness of such collections of cells and the variety of cell forms that may be associated together. Investigation shows that this is actually the case, and that thereby the correct interpretation of many of the particles visible when the powder is examined under the microscope is rendered a matter of some difficulty. In finely powdered leaves, for example, the cells of the palisade tissue and spongy parenchyma are often so broken up as to be scarcely recognisable. The vessels and sclerenchymatous fibres are better preserved, and can usually be identified. The epidermal cells, held together by the resistant cuticle, occur in small plates, while the collenchymatous tissue of the midribs is present in fragments, often several cells wide and thick. Hairs that are composed of large thin-walled cells suffer the same fate as the parenchymatous cells, but are more easy to identify, as they are covered by a very thin cuticle that can be stained by appropriate reagents.

In the present case, the examination of transverse and

FIG. 61.—Structural Details of Senna Leaf. $\times 370$. (Meyer.)

1. Lower epidermis, surface view; *a*, *b*, *c*, stoma with upper, middle, and lower part respectively in focus; *d*, scar of hair (in glycerin after chloral hydrate).
2. Section of epidermis with stoma; *sch*, mucilage (chloral and glycerin).
3. Hair.
- 4, 5, 6, 7. Vertical rows of cells with calcium oxalate crystals, from the midrib, in various positions. The calcium oxalate has been dissolved from 5, 6, and 7 by acid.
8. Fragment of a sclerenchymatous fibre of midrib, isolated by potassium chlorate and nitric acid.
9. Spiral vessel from one of the smaller veinlets.
10. Fragment of pitted vessel from midrib.
11. The same from a veinlet.
12. Fragment of spiral vessel from midrib.
13. Termination of a veinlet, from surface section. *a*, sclerenchymatous cell; *i*, intercellular space.
14. Cell containing a rosette crystal of calcium oxalate; from the powder.
15. Epidermal and palisade cells of upper surface, transverse section (glycerin after chloral).
16. Palisade cells of under surface.
17. Surface view of spongy parenchyma; *J*, intercellular spaces.
18. Collenchymatous cells from the midrib, longitudinal aspect.
19. Palisade cell, with contents of unknown nature.



longitudinal sections, and the investigation of the epidermis and appendages borne by it, give a sufficient knowledge of the structure of a leaf to allow of the student proceeding to the examination of the powder. This examination is, in itself, a check upon the examination of the leaf, for every cell and cell-content present in the leaf must be present in the powder, and, conversely, every cell and cell-content in the powder must be derived from and be present in the leaf. The student should, therefore, examine the powder for the more important elements, &c., that have been observed in the leaf, and should also satisfy himself that there is no element in the powder which he cannot identify with one in the leaf.

Coarse Powder.—Dry some senna leaves in a warm drying chamber (or over quicklime) and reduce them in an iron mortar to a coarse (about No. 20 to 40) powder. Sift this coarse powder first through a No. 20 and then through a No. 60 sieve, so as to separate the coarse particles (those that remain on the No. 20 sieve) from the medium (those that remain on the No. 60 sieve) and from the fine (those that pass through the No. 60 sieve).

Examine the coarse powder first. Spread it on a sheet of paper, and examine it with a hand lens; pick out fragments with a damp brush for section-cutting. If there is any difference, pick out the pieces that show this and examine them separately. Take pieces of the midrib as well as of the lamina. Treat them as follows:

(1) Fix some on elder-pith previously cut and moistened with gum and glycerin; place the fragments, if possible, so as to cut the veins transversely; put them in a warm place for few minutes until nearly dry. Then adjust the other half of the pith, press together, and cut sections. Transfer the sections of pith with the sections of leaf adhering to them to alcohol, then to a slide; moisten with water, and mount in chloral hydrate, or place the pith with the sections in chloral hydrate and warm gently.

By such means sections may easily be obtained; with a little care even that which passes through a No. 20 sieve but remains on a No. 60 may be so treated.

(2) Warm some of the fragments in chloral hydrate, and examine. Should this method make the leaf transparent enough, the characters of the epidermis, &c., may be determined.

(3) Mount one or two fragments in solution of potash.

Warm until the liquid boils gently, and cool. Press the cover-slip firmly down and at the same time slide it along. By this means the epidermis may often be detached when chloral hydrate fails to make the leaf sufficiently transparent.

Medium Powder.—Next examine the fragments of medium size. Rub about 0.2 gramme with 10 c.c. of solution of chloral hydrate in a small mortar and transfer to a test-tube, or, better, to a centrifuge tube. Warm in a water-bath for ten to twenty

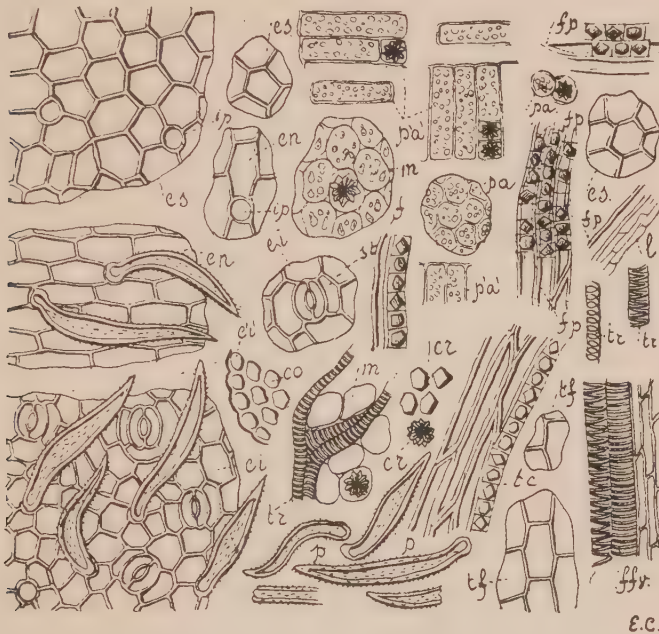


FIG. 62.—Powdered Senna. $\times 240$. *co*, collenchymatous cells; *cr*, prismatic and cluster crystals; *ei*, *e'i'*, lower epidermis; *en*, neural epidermis; *es*, upper epidermis; *ffv*, debris of fibrovascular bundles; *f*, *ffp*, lignified fibres from midrib or veins; *ip*, scar of hair; *l*, bast; *p*, hair; *pa*, *p'a'*, palisade cells; *st*, stomata; *tc*, cells with calcium oxalate; *tf*, cortical tissue of midrib. (Greenish and Collin.)

minutes, remove, and allow the fragments to subside, or, far better, separate them by centrifugation. Pour off the supernatant dark-coloured liquid, and transfer a little of the deposit to a slide. Add a drop or two of solution of chloral hydrate and examine. The digestion with the chloral hydrate will have removed most of the colouring matter and made the fragments transparent.

Many of the fragments present their surface view, many the

section ; both may be compared with the sketches of senna and the coarse powder. Numerous fragments of veins will also be found, the pericyclic fibres with their accompanying crystal cells being especially conspicuous. The details in this preparation are usually very clear.

Fine Powder.—Lastly, examine the fine powder ; this will contain the majority of the small fragments of parenchymatous cells, the isolated crystals from the pericyclic fibres, sand, &c., all of which will pass through the 60 sieve. In addition there will be present such small fragments of the leaf as can pass through the sieve.

Mix about 0·2 gramme with 10 c.c. of solution of chloral hydrate and warm for five minutes ; this suffices to clear and decolourise the fine powder. Allow it to deposit, or separate it by centrifugation. Groups of pericyclic fibres accompanied by the crystal cells are conspicuous ; portions of the epidermis are easily found ; hairs are more or less numerous, and many isolated crystals of calcium oxalate are also to be found. Most of the delicate parenchymatous cells of the palisade and spongy parenchyma are reduced to fragments that exhibit no distinctive features.

Next reduce a little of the original drug to fine powder ; take about as much in bulk as a white mustard seed ; moisten with alcohol, and, when nearly dry, add chloral hydrate, cover, and examine. The particles of powder will probably be sufficiently decolourised in a few minutes. Examine, and compare with the previous examination.

This preparation serves as a check upon the foregoing, and is also useful in affording information as to the relative proportion in which each of the various tissues is present.

This examination may also be supplemented by a preparation in water and another in dilute glycerin, in which, however, the tissues are by no means so clear.

Examination of the Fine Powder of Commerce.—Purchase some powdered Tinnevely senna, and examine it with the object of :

- (1) Identifying it as senna :
- (2) Ascertaining its freedom from other leaf powders.

Moisten about 0·2 gramme with a little dilute glycerin, and allow it to stand twelve to twenty-four hours. Examine a little on a slide ; the particles should have a fine green colour, and

there should be nothing of the nature of added starch grains, sand, &c.

Treat some of the powder as directed above for fine powder. Recognise and identify senna by the following characters :

Diagnostic Characters.—(a) The stomata, which are bordered by two cells with their long axes parallel to the ostiole ; (b) the one-celled, thick-walled, warty hairs ; (c) the pericyclic fibres with their accompanying crystals ; (d) the isobilateral structure.

Portions of the powder showing these characters, should be sketched and the sketches compared with those made from the leaf itself.

Mount a little in chloral hydrate, and compare with the powder of the genuine leaf ; the various tissues, &c., should be present in about the same relative proportion.

Lastly, examine the powder to ascertain whether any foreign powder is present. Focus and examine every particle in the field of the microscope ; it should be possible to assign to every one its position in the leaf, and impossible to find any particle differing so markedly from any of the tissues of the senna leaf as to leave little doubt of its being of foreign origin. Repeat this examination several times.

Buchu Leaves

Source.—The leaves of *Barosma betulina*, Bart. and Wendl.

Preparation of Sections.—Prepare a leaf for cutting by exposing it to a moist atmosphere for three or four hours. Cut transverse sections of the midrib as directed on p. 96, and place them in alcohol.

Examination.—Examine one of the thinnest in glycerin. The structure is not very distinct, but the following particulars can be made out :

The outer walls of the cells of the upper epidermis are very thick, transparent, and homogeneous ; the epidermal cells themselves have small cavities in which granules are visible ; below the cavities of each cell there is another thick transparent wall.

Allow water to flow on ; the inner wall gradually swells and becomes invisible or nearly so ; the swelling is most marked in the epidermis of the lamina between the midrib and the margin, and is so great as to lift the epidermis from the

remainder of the tissue, rupturing the vertical cell walls. The swelling is due to mucilage.

Mount a section in a drop of solution of ruthenium red in lead acetate. The mucilage swells, and is coloured pink. Some of the cells of the lower epidermis also colour red, showing that they too contain mucilage. Less striking red colourations may be observed in the bast and in the walls of part of the parenchymatous tissue.

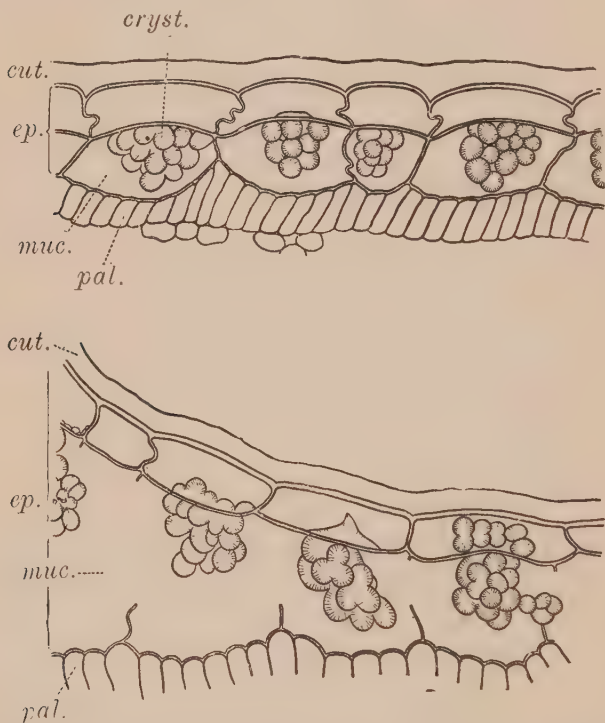


FIG. 63.—Buchu Leaf, transverse section of epidermis before and after the addition of water. *cryst.*, sphæro-crystalline masses of hesperidin; *cut.*, cuticle; *ep.*, epidermis; *muc.*, mucilage; *pal.*, palisade. $\times 350$.

Mount a section from alcohol in alcoholic solution of methylene blue (0.1 gramme methylene blue, 25 c.c. 95 per cent. alcohol) and after a minute or two allow a solution of methylene blue in glycerin to run on (0.2 gramme methylene blue, 10 c.c. alcohol, 40 c.c. glycerin); the mucilage is coloured blue.

The examination of sections treated in this way would lead one to suppose that the mucilage was deposited in a layer of

cells below the epidermis ; this, however, has been shown not to be the case. It is deposited on the inner surface of the lower wall of the epidermal cell itself in a manner analogous to that described for senna (see p. 102).

Mount a fresh section in water ; examine the granules in the epidermal cells, disregarding the mucilage ; they appear crystalline. Irrigate with solution of potash ; they dissolve with yellow colouration (reaction of hesperidin). Examine them more in detail from surface preparation, as described below.

Examine the outer wall of the epidermal cell ; it appears to consist of two layers, the outer of which (cuticle) bears curious little protuberances. Irrigate with chlorzinciodine. The cuticle stains yellow, the inner layer violet.

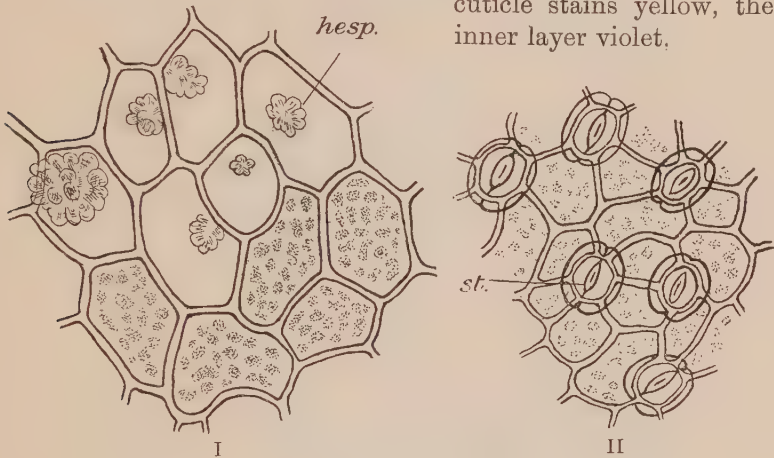


FIG. 64.—Buchu Leaf. Upper and lower epidermis. $\times 250$.

Examine the cells of the lower epidermis ; they resemble those of the upper but are smaller, and scattered among them are stomata. The palisade cells contain granular matter, among which minute starch grains can be detected by the iodine reaction. In the palisade and in the spongy parenchyma there are rosette crystals of calcium oxalate, but no single crystals can be detected.

Surface Preparations.—Soak a leaf in water, and when the mucilage has swelled insert a needle under the epidermis of the upper surface and strip it off. Examine in water ; if much air is entangled in the section get rid of it by one of the means alluded to on page 64. Observe first the cells : they are polygonal, and measure about 60 to 90 μ long and 40 to 60 μ wide. Their surface appears faintly and coarsely granular ; this is due

to the small protuberances mentioned above. There are no stomata.

Examine the distribution of the hesperidin. Much of it is in the form of sphaerites, but there are also radiating tufts as well as loose crystals. Almost every cell contains some.

To examine the epidermis of the under surface, cut thin surface sections and place in alcohol for half an hour or more.

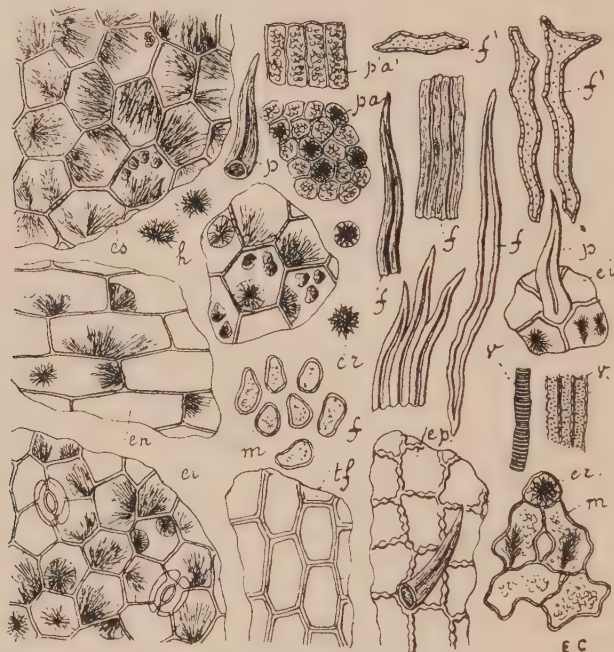


FIG. 65.—Powdered Buchu Leaves. $\times 240$. *cr*, calcium oxalate in rosette crystals; *ei*, lower epidermis with crystals of hesperidin; *en*, neural epidermis; *ep*, epidermis of petiole; *es*, upper epidermis; *f*, sclerenchymatous fibres; *f'*, fibrous cells from veinlets; *h*, hesperidin; *m*, mesophyll cells; *p*, hairs; *pa*, *p'a'*, palisade cells; *tf*, cortical tissue of midrib; *v*, vessels.

Transfer to water, and treat with solution of potash to remove the hesperidin.

The cells are polygonal, from 20 to 60 μ long, with walls that are not very thick. The stomata are very numerous, they are broadly oval, and are provided with a large ostiole. Here and there a group of a few smaller cells with thinner pitted walls can be distinguished; these are the cells above the oil glands.

The midrib can be examined in the usual way. The wood

of the meristele is fan-shaped, and the elements of which it consists are very small. Below the wood is an arc of bast, and below that again a crescent of pericyclic fibres.

Examination of the Powder.—Bearing in mind the constituents and the structure of the drug, the following method for examining the powder will prove satisfactory.

If it consists of a mixture of coarse and fine particles, separate these and examine them separately, as directed for senna. The fine powder should be treated as follows :

Mix a little with alcohol to remove air, and, when nearly dry, add

- (a) *Solution of ruthenium red in lead acetate*; the gelatinous masses of mucilage are easily detected by the pink colour they assume: they are distributed over the whole slide.
- (b) *Chloral hydrate*; the hesperidin can, after a few minutes, be distinguished as sphaerites in the epidermal cells; it is insoluble in water, alcohol, or chloral hydrate, but soluble with yellow colouration in caustic potash. The cuticle appears coarsely, but faintly, granular, and is often fissured (probably due to the contraction during the drying previous to powdering).
- (c) *Solution of potash*; the hesperidin dissolves, with yellow colouration.

The palisade cells are often in groups with the mucilage attached to them; the pericyclic fibres are in bundles, but they are free from crystals of calcium oxalate; the latter substance occurs in rosette crystals only, and is best seen in the potash preparation, as this is free from crystalline masses of hesperidin.

Tea

Source.—The leaves of *Camellia Thea*, Link.

Preparation and Examination of Sections. Infuse some of the leaves of Congou tea in boiling water twice in order to remove as much of the colouring matter as possible. Pick out some of the larger pieces that contain the midrib as well as the margin, and remove the superfluous moisture with blotting paper. Cut from some of the fragments small portions, taking care to include the margin, and put them into solution of chloral hydrate in a small wide-mouthed bottle. They should

remain a couple of days, or even longer, in this solution, and should then be used for surface preparations.

From other fragments cut transverse sections of the midrib as usual.

The leaf is bifacial, the epidermis of both surfaces is composed of small cells, and may bear long hairs; there are often two rows of palisade cells, but sometimes there is only one; the spongy parenchyma exhibits large air-spaces. In the centre of the leaf there are numerous cells containing calcium

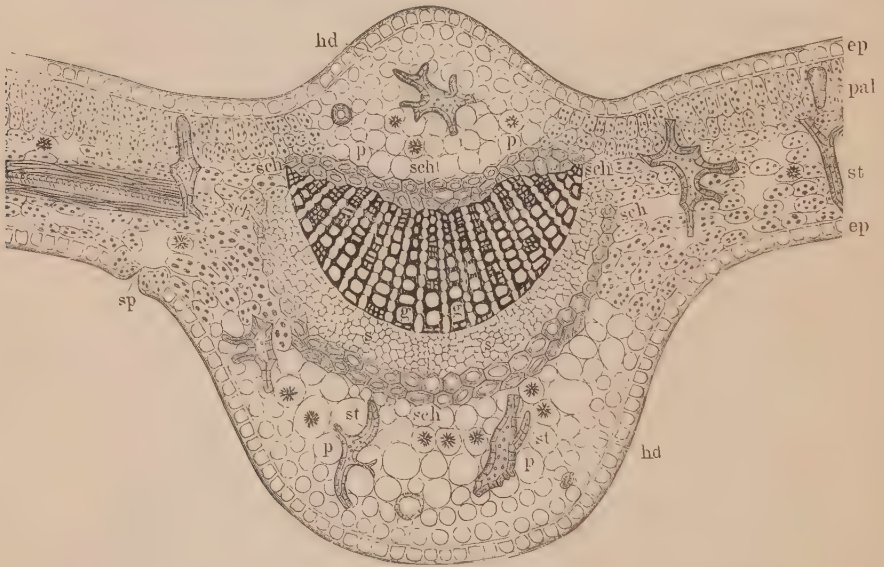


FIG. 66.—Tea, transverse section through the midrib. ep, epidermis; hd, hypodermis; g, vessels in wood; p, parenchyma; pal, palisade; s, sieve tissue; sch, sclerenchymatous fibres; sp, stoma; st, sclerenchymatous idioblasts. $\times 130$. (Warnecke.)

oxalate in varying forms, cluster crystals being often associated with small (sandy) crystals in the same cell.

In most varieties of tea there are remarkable sclerenchymatous cells (idioblasts) in the mesophyll. These cells are usually conspicuous in old leaves, or can easily be made so by staining with phloroglucin and hydrochloric acid, as their walls are strongly lignified. They may occur in the lamina as well as in the cortex of the midrib, but in young leaves (such as constitute Pekoe tea) they are found in the midrib only and are thin-walled, although also lignified. If any difficulty be

experienced in finding them, cut from the under surface of the leaf tangential sections of the midrib ; some of these will pass through the cortex, and exhibit the sclerenchymatous cells well.

Surface Preparation.—Examine next the leaf that has been soaked in solution of chloral hydrate, taking the under surface first. Observe the hairs. These are less abundant on old leaves than on young ones. They are often very long, many attaining $500\ \mu$ to $700\ \mu$ in length ; they are narrow, one-celled, and thick-walled ; they

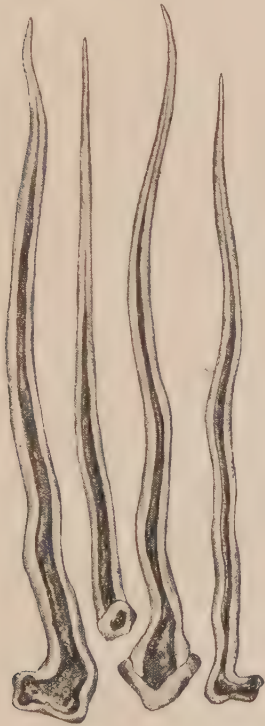


FIG. 67.—Hairs of Tea Leaf.
(Moeller.)



FIG. 68.—Tea Leaf, cleared by chloral hydrate, showing the veinlets, marginal tooth, and distribution of sclerenchymatous idioblasts, and calcium oxalate crystals. Slightly magnified. (Schimper.)

are bent nearly at right angles near the base, so as to lie almost flat on the surface of the leaf ; they are often surrounded at the point of insertion by radially arranged epidermal cells.

Next examine the epidermis. The cells are not very easy to see and require careful adjustment of the light ; if difficulty is experienced, cut surface sections, soak them first in chloral hydrate, then in glycerin, and finally transfer to water, or transfer them from chloral hydrate to a more dilute solution of the same reagent.

The cells are slightly wavy in outline, distinctly so in old leaves, not perceptibly so in young leaves. They often exhibit traces of the treatment they have undergone. The stomata are broadly oval, and exhibit a narrow ostiole; they are surrounded by three to four narrow, tangentially arranged cells. These stomata are characteristic, and should be carefully observed.

Lastly, examine the venation of the leaf and the teeth at the margin. Each tooth is a conical mass of parenchymatous

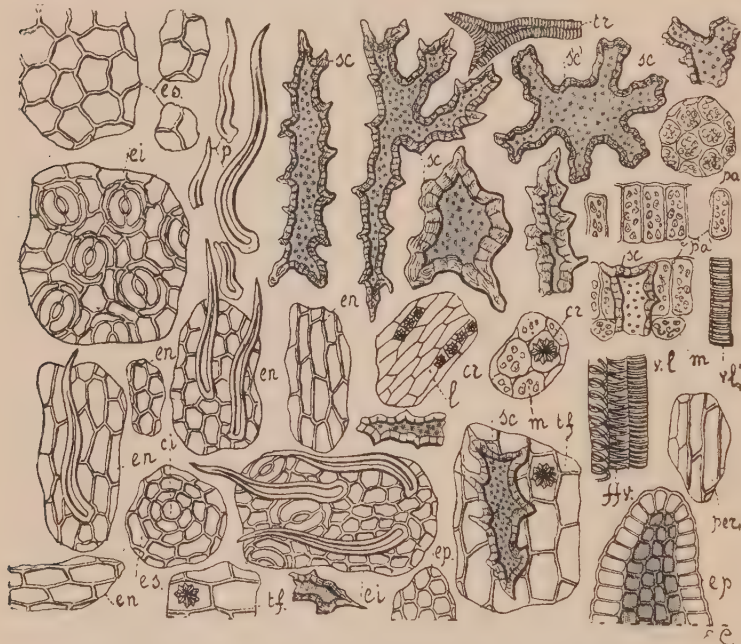


FIG. 69.—Powdered Tea. $\times 240$. *cr*, crystals; *ei*, lower epidermis; *en*, neural epidermis; *ep*, apex of marginal tooth; *es*, upper epidermis; *ffv*, debris of fibro-vascular bundles; *l*, bast with cluster crystals; *m*, spongy parenchyma; *p*, simple hairs; *pa*, *p'a'*, palisade cells; *per*, pericycle, slightly lignified; *sc*, idioblasts from the mesophyll and cortical tissue; *s'c'*, idioblasts from the pith of the stem; *tf*, cortical tissue; *tr*, tracheids; *vl*, vessel. (Greenish and Collin.)

cells, and often falls off, leaving, in old leaves, a brown scar. A veinlet runs up to this scar, and there generally spreads a little. The teeth and venation are characteristic.

The epidermis of the upper surface consists of small, delicate, polygonal cells, and exhibits no stomata.

Young leaves (Pekoe tea) are thinner and greener, and are more easy to clear and examine, than the older and darker

leaves of which Congou tea consists. The sclerenchymatous idioblasts are to be found in the midrib only, and the teeth are usually still attached.

The following characters serve to identify the leaf :

Diagnostic Characters :

- (a) The hairs, their shape and size together with the radiate arrangement of the cells at the base.
- (b) The crystals of calcium oxalate ; they are rosettes, and often accompanied by crystal sand.
- (c) The sclerenchymatous idioblasts, especially those of the petiole and midrib ; they are never entirely absent.
- (d) The stomata.
- (e) The teeth at the margin or the scars left by them.

Examination of the Powder.—Powdered tea may be examined as directed for powdered senna. Digestion with chloral hydrate and subsequent separation by centrifugation, repeated a second time, is strongly to be recommended. The final deposit can be washed free from chloral hydrate (separating by the centrifuge) and stained with appropriate reagents (e.g. phloroglucin and hydrochloric acid, by which the sclerenchymatous cells are at once made conspicuous).

Powdered tea may also be examined by moistening a little on a slide with alcohol, allowing it nearly to dry, adding chloral hydrate, and gently warming.

Stramonium Leaves

Source.—The leaves of *Datura Stramonium*, Linn.

Preparation and Examination of Sections.—Soften in the usual way. Cut and examine sections of the midrib, with part of the adjoining interneural portions.

The midrib is convex above and strongly convex below. The wood of the meristele has the shape of a rather flat arc ; it contains large vessels, often 30μ or more in diameter, and is provided with bast both above and below (a constant character in plants belonging to this order). The bast contains no sclerenchymatous elements, nor are there any pericyclic fibres.

The endodermis cannot be distinguished. The cortex is composed of large cells, some of which are filled with sandy

crystals of calcium oxalate; rosettes and prisms may also be found, but they are less frequent.

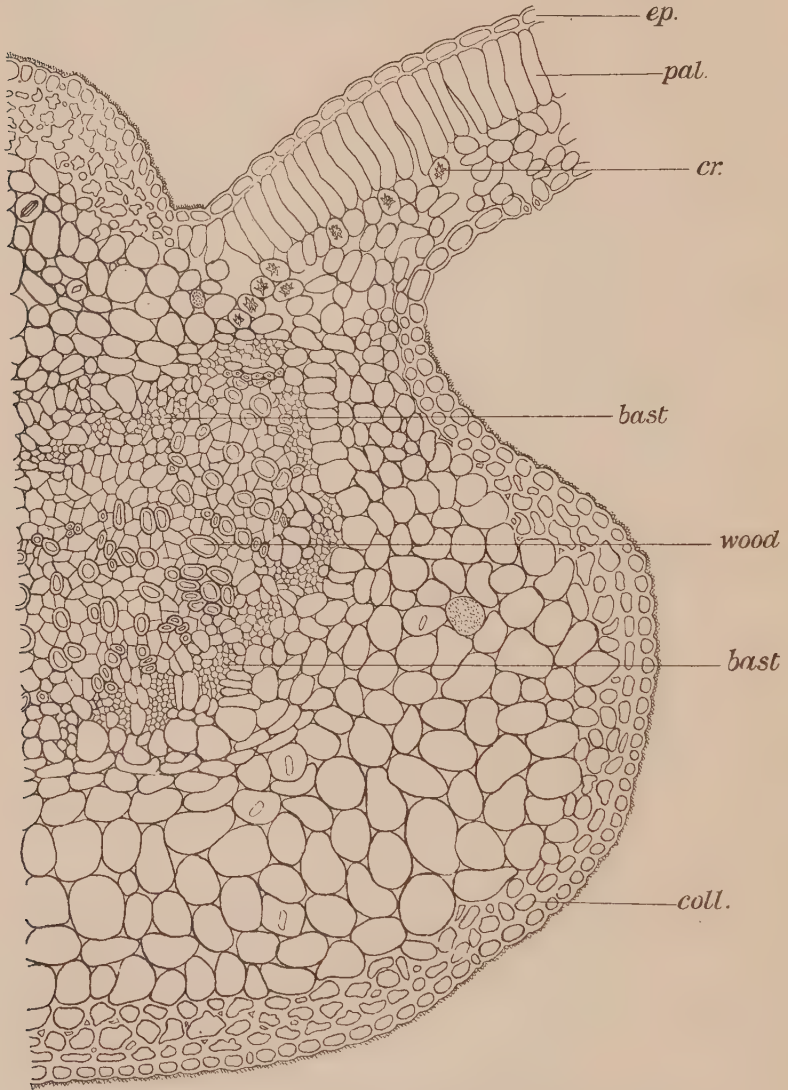


FIG. 70.—*Stramonium* Leaf, transverse section through the midrib. *ep*, epidermis; *cr*, cluster crystal of calcium oxalate; *coll*, collenchymatous tissue; *pal*, palisade. (After Tschirch.)

The interneural spaces exhibit an asymmetrical, heterogeneous mesophyll. The palisade cells are long and narrow,

and occupy about one half of the mesophyll. The spongy parenchyma is rather dense. Rosettes of calcium oxalate are present in abundance, they are situated chiefly in the spongy parenchyma abutting on the palisade; some prismatic crystals and occasionally a cell with sandy crystals may also be found.

Surface Preparations.—Warm some fragments of the lamina (between the stronger veins) in chloral hydrate on the water-bath for about half an hour. Examine in chloral hydrate. The features of the epidermis and the distribution of the stomata can generally be easily seen.

The epidermal cells vary considerably in size as well as in outline. For the same leaf the lower epidermis has cells with more wavy walls than the upper. Stomata are to be found on both surfaces; they are surrounded by three or four cells, one of which is smaller than the others, a common feature in Solanaceous leaves.

Two forms of hairs are present, simple and glandular; they are generally more numerous on young leaves and near the veins. In older leaves there are often but few to be found.

The simple hairs are uniserial and conical. They are composed of from three to five cells, the walls of which are warty and not very thick.

The glandular hairs are short, and consist of an oval pluricellular gland supported upon a pedicel.

Examination of the Powder.—Powdered stramonium is best prepared for examination by the method described under Powdered Senna (p. 110). The powder is liable to contain much sand; this appears largely in the deposit obtained by centrifuging gently for four or five seconds. The deposit obtained by centrifugation for fifteen to twenty seconds consists chiefly of the larger portions of the leaf together with a little sand.

Some fragments exhibit the surface of the leaf, but there are many that exhibit the section, and these can be easily identified by comparison with the sketches previously made, noting particularly the form and distribution of the calcium oxalate. The epidermis is, as a rule, not well seen even in those fragments that present their surface to the observer. Groups of axially elongated cells derived from the cortical parenchyma of the midrib are frequently to be found, the cells of which they consist being often of considerable size. Rosettes

of calcium oxalate are abundant, but pericyclic fibres are not to be detected. The vessels are often of considerable size.

The deposit obtained by centrifuging for sixty to one hundred and twenty seconds contains chiefly the smallest fragments; here portions of the epidermis are more numerous. Smaller fragments of the cortical parenchyma are present, and so are also rosettes of calcium oxalate.



FIG. 71.—Powdered Stramonium Leaves. $\times 240$. *cr*, crystals; *ccr*, crystal cells; *ei*, lower epidermis; *en*, neural epidermis; *es*, upper epidermis; *ffv*, debris of fibro-vascular bundles; *l*, bast; *me*, spongy parenchyma; *pa*, *p'a'*, palisade tissue; *pg*, glandular hairs; *po*, pollen grains; *pt*, simple hairs; *tf*, cortical tissue of midrib; *tr*, *v*, vessels, &c. (Greenish and Collin.)

The hairs, characteristic when present, are often so rare as to require careful search; they are usually more or less broken up, so that fragments only can be found (for a means of staining these compare powdered foxglove).

Mount also fresh preparations of the powdered leaf in chloral and in dilute glycerin.

Diagnostic Characters.--Diagnostic characters are to be found in

- (a) the characters of the transverse section, especially the calcium oxalate; such sections are always to be found;
- (b) the characters of the surface preparations, especially here also the calcium oxalate; the epidermis when visible;
- (c) the hairs, which are often very rare;
- (d) the size of the vessels of the wood and the cortical parenchyma of the midrib.

Coca Leaves

Source.—The leaves of *Erythroxylon Coca*, Lam.

Preparation and Examination of Sections.—Select for examination well-preserved Bolivian coca leaves. Prepare them for cutting by exposing them to moist air. Cut sections of the midrib at the proper point (see Bearberry Leaves), and treat them as directed for bearberry leaves.

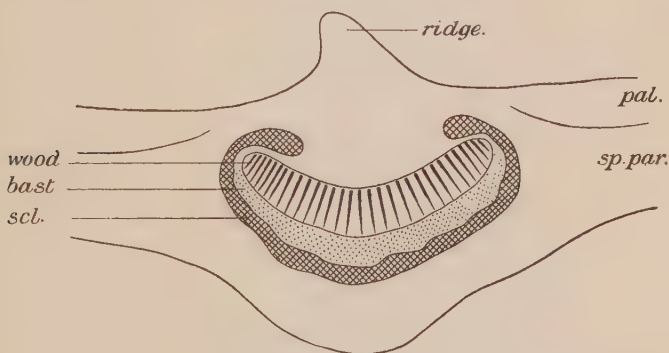


FIG. 72.—Bolivian Coca Leaf, diagrammatic section, showing the distribution of the tissues. $\times 65$.

The midrib exhibits no remarkable features; there is usually an interrupted ring of sclerenchymatous fibres present, but occasionally (some specimens of Java coca and of cultivated *E. Coca*, var. *novogranatense*) this is absent.

Above the midrib the ridge characteristic of Bolivian coca may be seen.

The epidermis of the upper surface calls for no remark. In

the palisade tissue observe occasional small prismatic crystals of calcium oxalate; in the spongy parenchyma rosettes of the same salt occur.

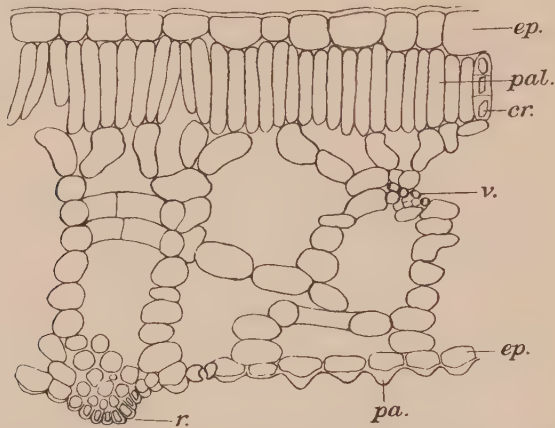


FIG. 73.—Bolivian Coca Leaf, transverse section. *cr.*, crystals of calcium oxalate; *ep.*, epidermis; *pa.*, papillose cells of lower epidermis; *pal.*, palisade; *v.*, veinlet. $\times 200$.

The cells of the lower epidermis are very remarkable and should be carefully examined; they are distinctly papillose, and at the apex of each papilla the cell wall is lenticularly thickened. Sometimes the cells contain mucilage (see Senna Leaves).



FIG. 74.—Coca Leaf. Lower epidermis. (Moeller.)

Prepare also transverse sections through the line that runs near the midrib from base to apex. The line is seen to be a ridge caused by the formation of several (usually five or six)

smaller, collenchymatous cells beneath the epidermis; their function is probably a mechanical one.

Surface Preparations.—Examine the epidermis as directed for bearberry leaves: observe minute granules and little rods on the cuticle (wax), and in each cell except those surrounding the stomata a clear disc surrounded by such granules; these discs are produced by the papillæ when viewed from above (see fig. 74). Note also that the stomata are bordered by four

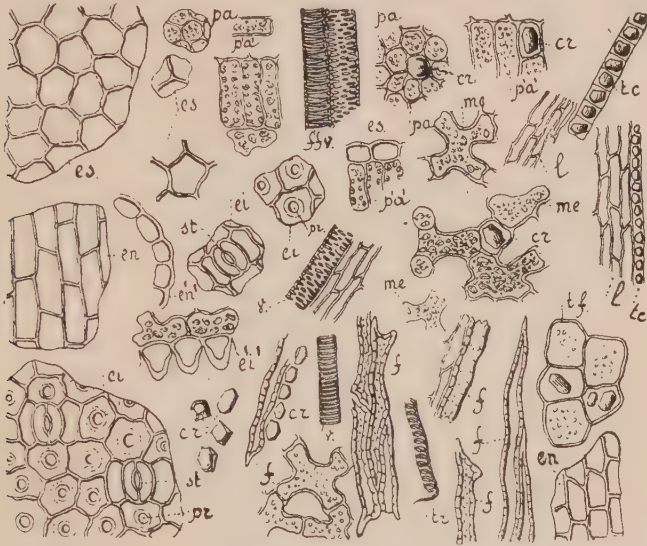


FIG. 75.—Powdered Coca Leaves. $\times 240$. *cr*, prismatic crystals of calcium oxalate; *ei*, lower epidermis, with surface view of papillose cells (*pr*); *ei'*, lower epidermis in section; *f*, sclerenchymatous fibres; *ffv*, fragments of vessels from midrib; *l*, bast; *me*, spongy parenchyma; *pa*, *pa'*, palisade cells; *st*, stomata, with two subsidiary cells parallel to the ostiole; *tc*, crystal cells; *tf*, cortical tissue of midrib; *tr*, vessels, &c.

cells, two of which have their long axes parallel to the ostiole.

Treat the leaf by warming with chloral as directed for senna; the circles on the epidermal cells are visible, but the granules have fused to minute globules; find and examine the curved line; note the axial elongation of the cells.

Some coca leaves also contain remarkable branched sclerenchymatous cells arranged parallel to the epidermis and closely applied to it. They are easily detected in surface

preparations, but may escape notice in sections. They are probably mechanical in function.

Examination of the Powder.—Examine this as directed for senna leaves.

Savin

Source.—The extreme tops of the twigs of *Juniperus Sabina*, Linn.

Preparation and Examination of Sections.—Soak some small twigs of savin, preferably those with small appressed leaves, for twelve hours in water. Cut transverse sections, and treat as usual.

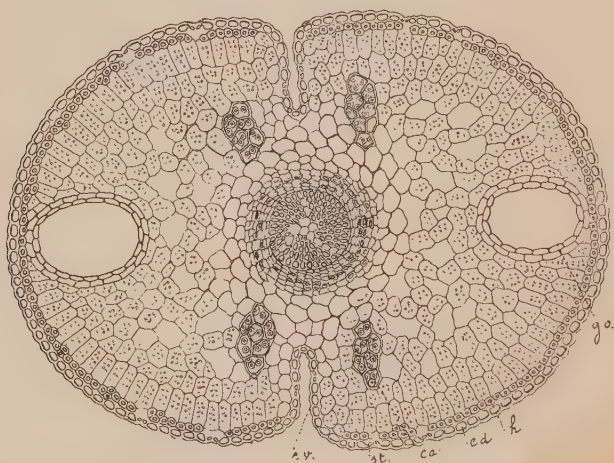


FIG. 76.—Savin, transverse section of twig. *ca*, cells with bordered pits; *ed*, epidermis of lower surface of adnate leaf; *ev*, epidermis of upper surface; *go*, oil gland; *h*, hypoderma; *st*, stoma. (Collin.)

The leaves are opposite and decussate, and the lower portion is usually adnate to the stem, the upper portion being free. On the under surface of each leaf a large oil gland can be seen. The sections cut may therefore be sections of the leaves without the stem, or sections of the small stem to which the two opposite leaves are attached.

The structure of the leaf is centric and the shape of the section nearly a semicircle. The meristele is embedded in the centre of a homogeneous mass of parenchymatous tissue; it consists of a small slightly arched group of tracheids with bast on their lower surface. The endodermis is not distin-

guishable, nor is there any fibrous pericycle, but on each side of the wood there is a small group of lignified cells with irregular thickenings (transfusion tracheids) ; these are very conspicuous when stained with phloroglucin and hydrochloric acid.

The epidermis consists of cells nearly square in section and provided with a thick cuticle. Stomata occur on both surfaces, but on the portion of the leaf that is free they are generally restricted to the upper surface.

Below the epidermis there is a hypoderma consisting of a single or sometimes double row of cells with small rounded outline and thickened walls. Embedded in the lower portion of the mesophyll is a large oil gland.

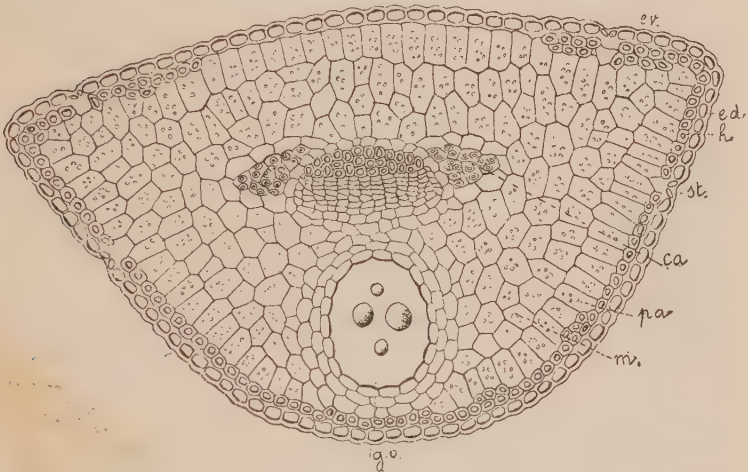


FIG. 77.—Savin, transverse section of leaf. *ca*, cells with bordered pits ; *ed*, epidermis of lower surface ; *ev*, epidermis of upper surface ; *go*, oil gland ; *h*, hypoderma ; *m*, mesophyll ; *pa*, palisade ; *st*, stoma. (Collin.)

Stain a section with phloroglucin ; observe that the wood and transfusion tracheids are strongly lignified, the hypoderma less strongly so. Examine minutely the stomata ; these also are lignified.

Maceration Preparation.—Digest a twig in solution of potash on a water-bath for fifteen minutes, wash in distilled water and tease out on the slide.

The epidermis is composed of cells with moderately thickened pitted walls. Near the stomata they are more or less isodiametric, but in other portions axially elongated.

Below the epidermis and closely adherent to it is the hypodermis; the cells can be teased apart, and are then seen to be long and narrow, with small lumen, blunt ends, and irregular outline.

Examine the stomata carefully. They are surrounded by four or five cells, which partly overhang the guard-cells. The latter are lignified, especially on their mutually apposed surfaces, and from the extremity of the stoma there appears a

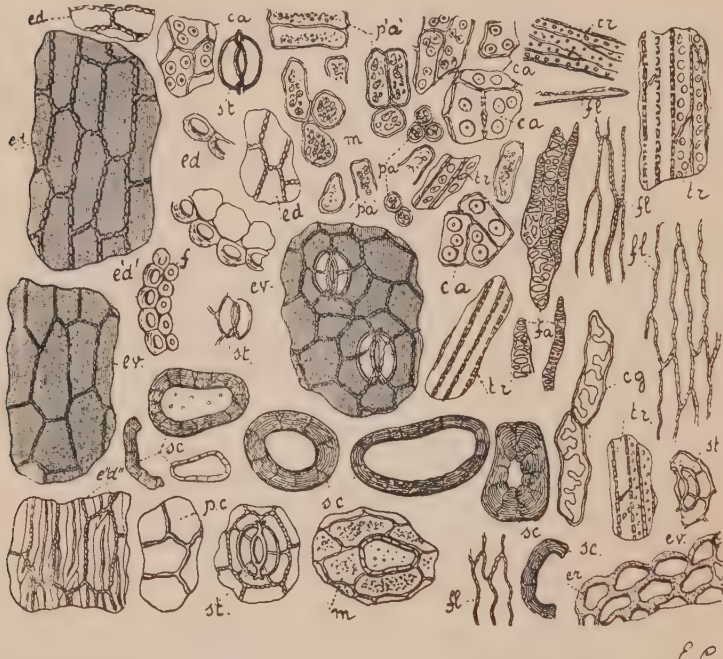


FIG. 78.—Powdered Savin. $\times 240$. *ca, cg*, cells with areolated pits and supports; *ed, ed'*, lower epidermis; *ed'd''*, the same with fibrous hypodermis attached; *ev*, upper epidermis; *fa*, reticulated fibrous cells; *st*, stomata; *tr*, tracheids; *m*, mesophyll; *sc*, sclerenchymatous cells (found only in *J. phoenicea*); *fl*, bast fibres from stem. (Greenish and Collin.)

small lignified projection (compare fig. 78); this appearance is very characteristic.

In the teased leaf groups of the transfusion tracheids are easily found.

Examination of the Powder.—Powdered savin is best prepared for examination by exhausting with chloral hydrate. The stomata and transfusion tracheids are very characteristic, and can be detected at once in a stained preparation. The

epidermis is also easily found and characteristic, but the details of the fibrous hypoderma are difficult to distinguish, as it adheres pertinaciously to the epidermis. The stomata, the transfusion tracheids, the epidermis, and the fibrous hypoderma render this leaf one of the easiest to identify.

Foxglove Leaves

Source.—The leaves of *Digitalis purpurea*, Linn.

Preparation and Examination of Sections.—For examination, select preferably foxglove leaves that are not very hairy on the under-surface; by this means the examination of the epidermis in surface preparations is much facilitated. Prepare them for cutting by exposing them to a moist atmosphere for twelve hours; at the same time soak some small fragments of the interneural lamina in chloral hydrate.

Cut and examine transverse sections of the midrib as usual. Observe that the wood is crescent-shaped, and possesses numerous large vessels. Below it is the bast, which is supported by a sheath of collenchymatous cells; a similar band of collenchyma is visible above. The cortex is composed of parenchymatous cells which, in transverse section, are rounded, but in radial section are (as is usual) axially elongated.

Examine the interneural spaces. The leaf is dorsiventral; the palisade cells are short and broad, and the mesophyll occupies about the same space as the palisade. The epidermal cells of the upper surface are comparatively large, those of the lower small; the latter surface exhibits stomata that are raised above the level of the epidermis. Both surfaces bear characteristic hairs. These are either simple (protective) or glandular. The former are uniserial and pluricellular; they consist of three or four thin-walled cells, and are either slightly warty or nearly smooth. Frequently the cells are collapsed.

The other kind of hair is glandular, consisting of a short pedicel supporting a one- or two-celled gland.

These and the stomata can be better examined in surface preparations.

In no part of the section can calcium oxalate be detected.

Surface Preparations.—The surface of the leaf can best be examined by warming in chloral hydrate in a water-bath for ten to fifteen minutes, or by allowing it to stand cold for

twelve to twenty-four hours. Cut out from the pieces all the larger veinlets until the fragments are only one or two millimetres in diameter.

The upper epidermis is usually very distinct, and is composed of polygonal cells with no stomata at all or only very few. The presence of the numerous hairs on the under sur-

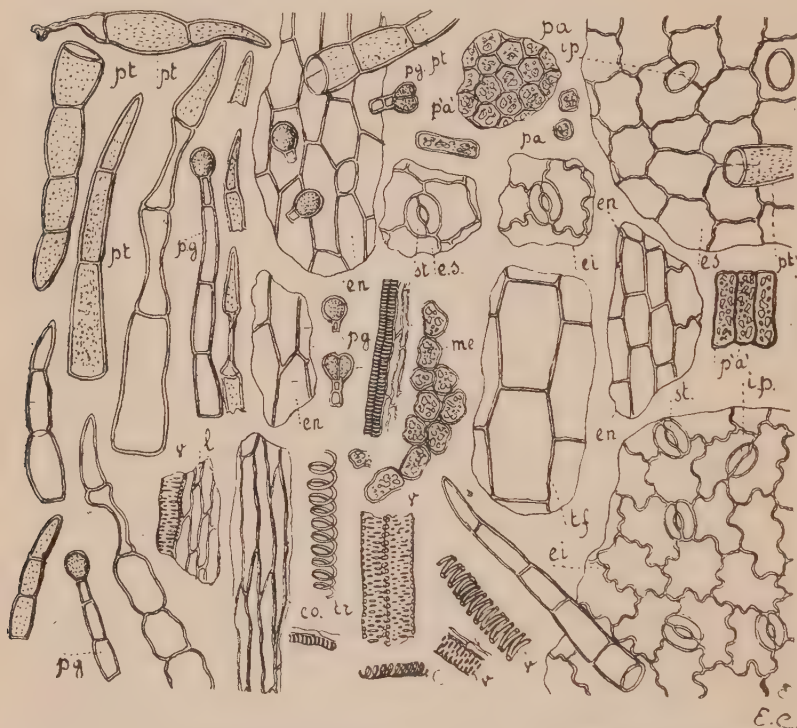


FIG. 79.—Powdered Foxglove Leaves. $\times 240$. *co*, collenchymatous cells of the midrib; *ei*, lower epidermis, cells with sinuous walls; *en*, neural epidermis; *es*, upper epidermis; *ip*, scar of fallen hair; *l*, bast; *me*, spongy parenchyma; *pa*, *pa'*, palisade cells; *pg*, glandular hairs; *pt*, simple hairs; *st*, stomata; *tf*, cortical tissue of midrib; *tr*, *v*, vessels, &c. (Greenish and Collin.)

face rather obscures the field, but under tolerably favourable conditions there is little difficulty in observing the wavy outline of the epidermal cells and the presence of stomata. The hairs may also be examined in this preparation.

Examination of Foxglove Powder.—Treat the powder as directed for stramonium.

The first deposit contains the larger fragments, together with any sand that may be present. Conspicuous in the preparations are pieces of the cortex of the midrib and stronger veins; these are easily identified if they still have the epidermis adhering, as this will exhibit the broken bases or the scars of hairs. Fragments of the smaller veins are also very numerous; these generally have the epidermis attached and can thus be recognised; they are also seen to be free from pericyclic fibres and from calcium oxalate. Some of these fragments include portions of the interneural mesophyll, which is likewise free from calcium oxalate. Pieces are also to be found showing their transverse section, but these are not so numerous.

Among the smaller fragments of cells are numerous portions of the broken hairs. These may be more readily detected when stained as follows:

Transfer a little of the deposit to a slide; carefully remove the chloral hydrate, wash with a drop or two of water (which also remove), and add two or three drops of Soudan red in glycerin; cover and gently boil for a few seconds, cool, and, if necessary, add a drop of glycerin. Allow the preparation to stand for a few minutes before examining it. The cuticle and the hairs will stain red, the other cell walls acquiring at most only a slight colour. The fragments of hairs can thus be found without difficulty; most of them are warty, but some are smooth.

In the deposits obtained by longer centrifugation (thirty seconds) fragments showing the epidermis are more numerous, and the characters of that tissue can be identified.

Diagnostic Characters:

- (a) The wavy epidermal cells with small stomata;
- (b) The three-celled, simple hairs with thin, often warty walls;
- (c) The absence of pericyclic fibres;
- (d) The absence of calcium oxalate.

Belladonna Leaves

Source.—The leaves of *Atropa Belladonna*, Linn.

Preparation and Examination of Sections.—Prepare and examine belladonna leaves as directed for foxglove. Observe that the meristele contains bicollateral bundles. The mesophyll is

characterised by the presence of large cells packed with minute (sandy) crystals of calcium oxalate; under the low power this appears as a dark granular substance.

The epidermis may be examined either as directed for fox-glove or by warming for fifteen minutes on a water-bath with

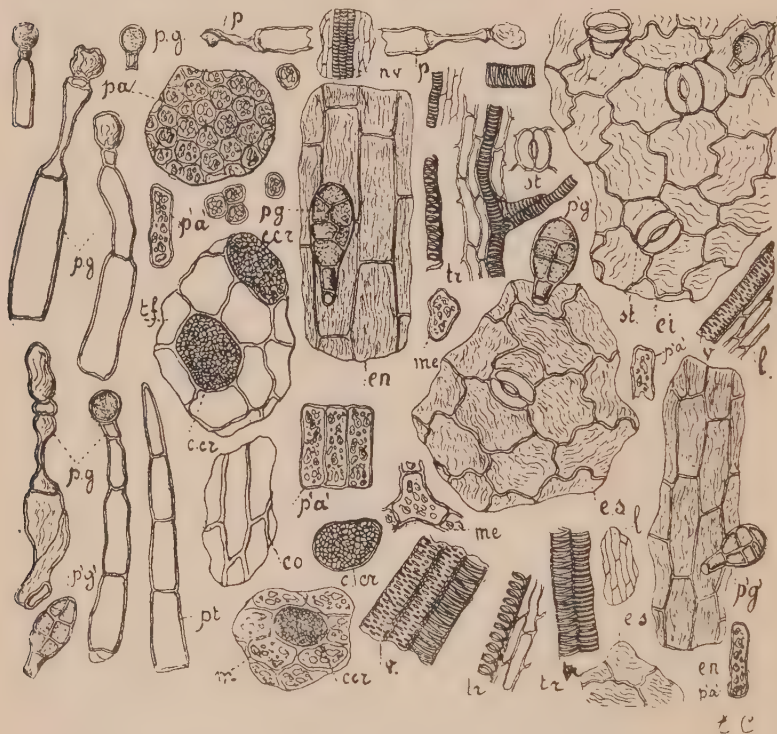


FIG. 80.—Powdered Belladonna Leaves. $\times 240$. *ccr*, cells with sandy crystals of calcium oxalate; *co*, collenchymatous cells from cortical tissue of midrib; *ei*, epidermis of under surface; *en*, epidermis over the veins, with striated cuticle; *es*, epidermis of upper surface, with striated cuticle and occasional stomata; *l*, bast; *me*, branching cells of spongy parenchyma; *nv*, fragments of small vein; *pa*, *p'a'*, palisade cells; *pg*, glandular hairs, long and short, with unicellular and pluricellular glands; *st*, stomata surrounded by three or four cells, one of which is smaller than the others; *tf*, cortical tissue of midrib; *tr*, *v*, vessels, &c. (Greenish and Collin.)

solution of potash (about 1 per cent.), and stripping the epidermis; both methods give good results, but the former is perhaps simpler.

Note the large epidermal cells with wavy walls and striated cuticle; the stomata are also large, and are situated

principally on the under surface. Hairs may be occasionally found. They are of two kinds, simple or glandular. The former are uniserial, and consist of two, three, or four thin-walled cells. The latter either resemble the simple hairs with the exception of the terminal cell, which is glandular, or they consist of a short pedicel bearing a pluricellular gland.

Diagnostic Characters :

- (a) The large epidermal cells, with wavy walls and striated cuticle.
- (b) The stomata, surrounded by three or four cells, one of which is smaller than the others.
- (c) The cells filled with sandy crystals of calcium oxalate.
- (d) The absence of pericyclic fibres.
- (e) The bicollateral bundles.

Henbane Leaves

Source.—The leaves of *Hyoscyamus niger*, Linn.

Preparation and Examination.—Prepare and examine henbane leaves as directed for belladonna.

The epidermis of both surfaces is composed of cells with very wavy walls and smooth cuticle; they vary from 40 to 100 μ in length. Both surfaces bear simple and glandular hairs, as well as stomata. The latter are broadly oval, and surrounded by three or four cells, one of which is smaller than the others; this arrangement is commonly met with in Solanaceous plants. The stomata average about 23–27 μ in length. The simple hairs are uniserial and conical, and have thin walls. The glandular hairs are usually long, uniserial, and terminated by a small bicellular gland, or by a large ovoid pluricellular one.

The mesophyll is heterogeneous and asymmetrical; the cells of the spongy parenchyma often contain prismatic crystals of calcium oxalate; in this respect henbane differs conspicuously from stramonium and belladonna, which contain chiefly cluster crystals and sandy crystals respectively.

The midrib is biconvex. The wood is curved, and has bast both above and below it, bicollateral bundles being constant in the natural order Solanaceæ. Neither bast nor pericycle contains any lignified elements.

The diagnostic characters of powdered henbane leaves are :

- (a) The remarkable glandular hairs ;
- (b) The calcium oxalate, mostly in prisms ;
- (c) The epidermal cells with wavy walls ;
- (d) The stomata surrounded by three or four cells, one of which is larger than the other.
- (e) The absence of pericyclic fibres.

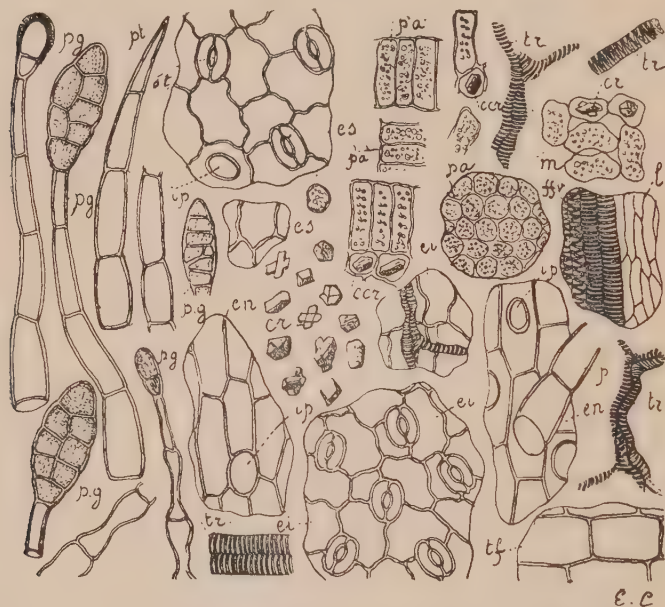


FIG. 81.—Powdered Henbane Leaves. $\times 240$. *ccr*, crystal cells; *cr*, crystals of calcium oxalate; *ei*, lower epidermis; *es*, upper epidermis; *ffv*, portion of fibrovascular bundle of midrib; *ip*, scar of fallen hair; *m*, spongy parenchyma; *pa*, *p'a'*, palisade cells; *pg*, glandular hairs; *pt*, simple hairs; *st*, stomata; *tf*, cortical parenchyma of midrib; *tr*, tracheids and vessels. (Greenish and Collin.)

Identification of an Unknown Powder as that of a Leaf

Having now examined several leaves and their powders, the student must inquire by what means he may identify an unknown powder as that of a leaf.

The following are the most reliable characters of a leaf powder :

- (1) The presence of an epidermis with stomata, hairs, &c.
- (2) The presence of palisade tissue ;

- (3) The presence of abundance of chlorophyll or of the brown substance derived from it ;
- (4) The presence of veins and veinlets.

The identification of the botanical source of an unknown leaf is extremely difficult unless the leaf happens to be one frequently used as a food or a drug. Solereder¹ has given a large number of accumulated facts which may be of service in this respect.

Finally, it may be observed that if a powder has been prepared from leaves only the following tissues or cell contents should be absent :

- (1) Cork cells (with few exceptions, such as eucalyptus leaves) ;
- (2) Aleurone grains ;
- (3) Fat and reserve starch (with few exceptions) ;
- (4) Large proportion of lignified tissue ;
- (5) Large, thick-walled vessels.

¹ *Anatomie der Dicotyledonen.*

SECTION VIII

BARKS

INTRODUCTION

THE structure of the wood and also that of the young stem in which no very far-reaching secondary changes have been produced has been considered in Sections V. and VI. The student should now direct his attention to the bark. Before doing so it is very desirable that he should study the section of his text-book of botany that deals with the formation of this part of the stem.

It will be convenient here to use the term 'bark' in its widest signification, and to understand by it all those tissues of the stem that are exterior to the cambium, no matter whether they are primary, secondary, or tertiary—that is, whether they have been produced by the division of the cells of the growing point, or whether they have been produced from a cambium (secondary tissues), or from a secondary cambium (tertiary tissues).

The changes by which the bark is developed from the tissues of the young stem are brought about largely by the activity of two circles of merismatic cells, an inner circle or cambium and an outer circle or phellogen (cork cambium). The cambium produces wood towards the interior of the stem and bast towards the exterior; the phellogen produces cork towards the exterior and usually phelloderm towards the interior, but the latter tissue is often developed sparingly or not at all.

The former of these two circles invariably develops between the primary bast and primary wood, but the point at which phellogen forms is subject to considerable variation. Sometimes it is the epidermis, frequently the sub-epidermal layer of cells, but it may also form in any layer of the cortex,

in the endodermis, or in the pericycle. As the formation of cork cells by the phellogen causes the tissue exterior to it to perish and often disappear, it is evident that the structure of the bark may thereby be considerably modified.

Much change may also be induced by the formation of secondary phellogens. These may arise in the phelloderm, in the cortex, or in the secondary bast, and, as with the primary phellogen, their production results in the death and ultimate

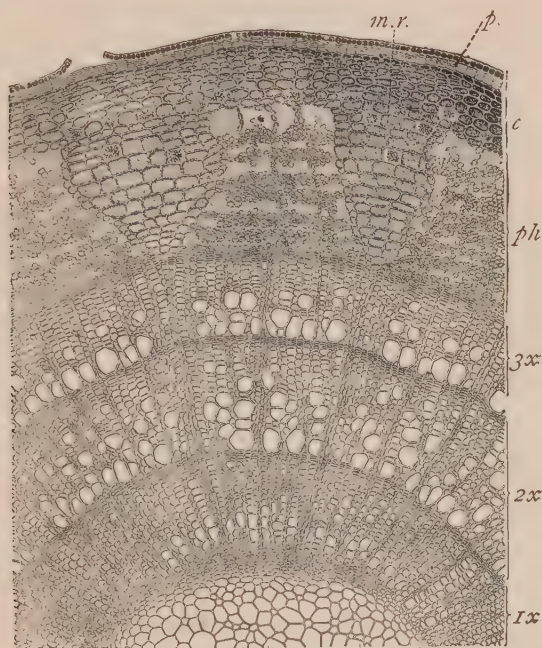


FIG. 82.—Transverse section of a twig of lime, three years old. 1x, 2x, 3x, the successive annual rings of wood; *ph*, phloem (bast); *c*, cortex; *m.r.*, primary medullary ray; *pe*, periderm (cork). (After Kny.)

destruction of the tissues external to them. The mass of external tissue thus formed includes all the layers of cork together with varying quantities of cortical tissue, pericycle, and bast. It has been called by botanists 'bark,' but as the term is in common use to designate all the tissues of the stem exterior to the wood, this mass of external protective tissue, the *outer* portion of the bark, may be specified as 'outer bark.'

In the examination of a bark the following tissues may therefore be met with :

- | | |
|-----------------|--------------------------|
| (1) Cork. | (4) Cortex. |
| (2) Phelloderm. | (5) Bast (or bast ring). |
| (3) Outer bark. | |

In order to be able to describe a section of a bark correctly it is necessary to know how these tissues may be recognised and delimited.

Were an easily recognisable endodermis present, as is the case with many herbaceous stems, it would be very desirable

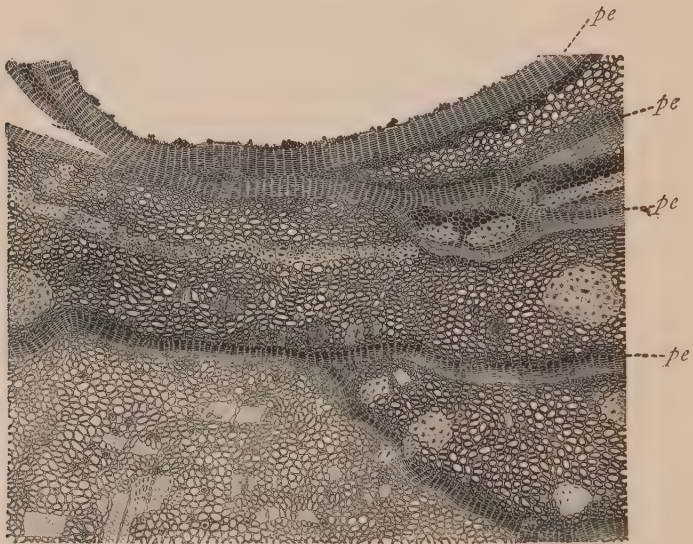


FIG. 83.—Outer Bark of Oak, transverse section. *pe*, periderm (cork) layers arising at different depths in the cortex. (After Kny.)

to identify it, and thus at once determine the inner limit of the cortex and the outer limit of the bast ring. Unfortunately, the endodermis is seldom to be found ; in many barks it has been thrown off by the formation of secondary phellogens within it ; in others the growth of the bark has rendered it indistinguishable. Recourse must, therefore, be had to other means of determining the inner limit of the cortex, and these, while often available, do not allow of the same precision as the identification of the endodermis would.

In the first place it must be observed that sclerenchymatous fibres frequently develop in the pericycle. These

fibres often differ in shape &c. from the fibres of the secondary bast. They are often designated 'primary bast fibres,' although they have not had their origin in the bast, but in the pericycle. Since the endodermis is immediately exterior to the pericycle, these pericyclic fibres mark, more or less accurately, the position of that tissue. Moreover, the cells of the pericycle often lignify and form a ring of sclerenchymatous cells, in which the pericyclic fibres are commonly included. Such a ring of sclerenchyma is found in oak bark, witchhazel bark, &c., and indicates the position of the endodermis in these drugs.

In many barks, however, no such ring of sclerenchyma or bundles of pericyclic fibres are to be found. In these cases it is advisable to trace the course of the medullary rays from the cambium towards the cork. As far as this can be done the tissue is undoubtedly part of the bast ring; but there always is to be found a region, usually narrow, which cannot be definitely assigned to either cortex or bast.

The student is advised to make a preliminary examination of the transverse section under a low power, and to make a diagrammatic sketch of it upon which the limitations of the tissues present should be indicated. He can then proceed to take each tissue in succession, to examine and describe it (compare the diagrammatic sketch of *Cascara* bark, fig. 84).

1. **Cork.**—The cells of which this tissue is composed are developed from a single layer of cells (phellogen) by repeated tangential division. They are consequently arranged in regular radial rows, an arrangement which is usually easily visible, even in cork of considerable age. They are often tangentially elongated, and have thin, strongly refractive walls. They often contain an amorphous brown or red substance that reacts for tannin, and their walls are also frequently yellowish brown in colour. The walls of cork cells are always suberised and very frequently lignified. In surface view they are polygonal and nearly isodiametric.

While these are the characters of typical cork cells, variations from the type often occur. In some barks the cell walls exhibit an appreciable thickening, which may be uniform or restricted to the outer or to the inner wall. In the cork of *cascarilla* bark crystals of calcium oxalate are embedded in the inner wall, a most unusual occurrence, and one therefore that materially aids in the identification of the drug.

Phelloderm (or Secondary Cortex).—This tissue is also developed from the phellogen, but on its inner surface. The cells of which it is composed are arranged, as cork cells are, in radial rows. They are, however, easily distinguished from cork cells by their walls not being suberised and by the absence of colouring matter, while the arrangement in radial rows distinguishes them, at least when young, from the cells of the cortex upon which they abut. When, however, the development of phelloderm is large, the radial arrangement of the cells becomes obliterated, and this tissue cannot then be distinguished from the cortex.

Cortex.—The cortical tissue, or primary cortex, to distinguish it from the phelloderm or secondary cortex, is composed of parenchymatous tissue, the cells of which are usually tangentially elongated and exhibit more or less well-marked intercellular spaces. Near the cork it often differentiates into strands of collenchymatous tissue. It often contains sclerenchymatous cells, either isolated or in groups; sclerenchymatous fibres are less frequently to be found. Secretory tissue of various kinds—such as oil cells, oil glands, &c.—may be present, and contribute to the identification of the drug. Among cell contents chlorophyll is often contained in the outer layers, while starch grains and calcium oxalate crystals are frequently to be found.

Bast Ring (including the Pericycle).—Abutting upon the cortex there is to be found in many barks a more or less continuous ring of sclerenchymatous cells, with which groups of fibres are often associated.

Next to the sclerenchymatous ring comes the bast ring or bast zone. This tissue is traversed by medullary rays continuous with those of the wood, and may therefore be conveniently divided into medullary rays and bast rays.

The medullary rays of the bast resemble in appearance and contents (starch, calcium oxalate, &c.) those of the wood, but their cell walls remain thin and do not lignify. Towards their outer limit they usually widen, and here often sclerenchymatous cells, or various forms of secretory tissue, such as oil cells, oil glands, &c., may be found.

The essential components of the bast rays are sieve tubes and bast parenchyma, but with these there may be also associated secretory tissue and sclerenchymatous cells or fibres.

Sieve tubes may be distinguished from bast parenchyma by

the difference in the size of the transverse section, and by the difference in the thickness of the wall. Usually the sieve tubes are larger, and have thicker and less regular walls, which also often exhibit a bluish colour. Sometimes the companion cell that is attached to the sieve tube can be detected, and when the tubes are of large size portions of the sieve plate can often be observed. Should these, as is commonly the case, be covered with callus, the latter can be stained with appropriate reagents. Digestion on the water-bath with solution of caustic potash so loosens the cells that the sieve tubes can by this means be isolated and examined, but it is only in comparatively few instances that they possess any decided diagnostic value, though they frequently afford important indications of the nature of a powder.

To sclerenchymatous cells and fibres, on the other hand, if present, considerable importance is to be attached. The forms of the cells, of their sections, the thickness of the walls, the extent to which lignification has taken place, should be ascertained, and the distribution of the cells should be studied. Sclerenchymatous cells are more commonly grouped, but the fibres may occur either isolated or in groups; in the latter case the arrangement of the groups must be noted.

It is very desirable that the student should be quite familiar with the characters that distinguish sclerenchymatous fibres from sclerenchymatous cells, both in transverse and radial sections and also when isolated. The following characters should be carefully noted:

In transverse section sclerenchymatous fibres are usually rounded or polygonal, and much thickened, so that the cavity is often reduced to a point; they are comparatively rarely oval and pits are seldom visible. Sclerenchymatous cells, on the other hand, are often irregularly rectangular in shape, have thick, striated walls, which are traversed by branching pits; the cavities are sometimes large, but if much reduced they are usually linear, not punctiform.

In radial section, and also when isolated, typical fibres are distinguished by their great length and tapering ends; the pits are scattered, and have the form of narrow slits, usually arranged in a left ascending spiral. Sclerenchymatous cells have much the same shape in radial as in transverse sections; they have square or rounded ends, and circular pits.

Although these characters sufficiently distinguish typical

sclerenchymatous cells from typical fibres, intermediate forms are not unfrequently found, as, for instance, in cinchona bark. Such intermediate forms may be referred to the class of fibres if their pits are narrow and oblique, to the cells if they are rounded, but in each case the elements in question should be accurately described.

It sometimes happens that in the older (outer) portions of the bast ring the sieve tubes that have ceased to be active are pressed by the growth of the bark into strands in which little or no structure can be observed. As the sieve tubes often assume a tangential arrangement, these strands of collapsed sieve tubes are often prolonged to a considerable extent tangentially, and may be very conspicuous in the transverse section of the bark (canella, cinnamon, &c.).

Outer Bark.—When this tissue is present it is usually dark brown in colour, and consists of dead portions of the cortex or bast ring, alternating with layers of cork. The elements of these respective tissues may readily be identified, although somewhat modified by the changes they have undergone.

Diagnostic Characters.—The following are the chief features to which the student should particularly direct his attention as being of considerable diagnostic value.

1. *The Cork.*—The extent to which this tissue is produced varies in different barks. Important details are to be found in the size of the cells and the nature of the walls; whether the latter are thin or thick, and whether the thickening, if present, is uniform on all the walls. The nature and colour of the contents should also be noted.

2. *The Phelloderm.*—In so far as this tissue is distinguishable from the primary cortex it seldom possesses any distinctive features. In canella bark the cells of the phelloderm are characterised by their very pronounced one-sided thickening.

3. *Cortex.*—The parenchymatous cells of the cortex do not, as a rule, present any remarkable features, but their contents may (starch, calcium oxalate, &c.). More important is the presence of secretory tissue of any kind (oil cells, oil glands, &c.) or sclerenchymatous cells or fibres. Any of these should be subjected to careful scrutiny.

4. *Bast Ring.*—This tissue usually constitutes the major part of commercial barks, and it is in it that distinguishing characters are usually found.

The presence or absence of sclerenchymatous cells or fibres should first be noted. If either be present, the shape of the cells, the thickness and nature of the walls, the striations, pits, &c., should be studied. The amount and distribution of these elements should also be examined; whether they are isolated or in groups, and in the latter case whether the groups are radially or tangentially arranged. While these characters are valuable for the entire drug, they are less valuable for the powdered drug; in the latter case the details of the individual elements are necessary.

The presence or absence in the bast ring of any form of secretory tissue is also most important. The shape of starch grains that may be present, the form assumed by the calcium oxalate, all contribute their quota to the identification of the drug. The characters of the sieve tubes, their distribution, size, the position of the sieve plates, &c., should also be determined.

Powdered Barks.—Although the various official barks exhibit in section notable differences, these differences frequently depend upon the arrangement of the tissues and elements present rather than upon any characters distinctive of the cells themselves. These differences in arrangement are naturally lost in the powdered drugs, and it becomes necessary to devote minute study to the details of the cells that are present.

Perhaps the most important of all these is the bast fibres. These resist the action of the grinding machinery, and, though they may be and often are broken, they usually exhibit their longitudinal aspect, and are easy to detect and identify. Their size and thickness of wall are occasionally valuable, as are also the character of the pits, the striation, if any, of the walls, and their colour. The number in which they are present should also be observed.

Next in importance to the bast fibres are the sclerenchymatous cells. They commonly resist the action of the drug mill, and are found in the powder in a more or less intact state, either isolated or two or three together. Sometimes here also the character of the secondary thickenings and other details may be useful.

The same may be said of the sieve tubes, the presence of which is in itself useful as an indication of the nature of the

powder. Especially in the coarser particles of powder they may be found without great difficulty, particularly if the precaution is taken to bleach the powder and stain the callus plates. The size of the sieve tubes and the arrangement of the sieve plates, whether transverse or oblique, simple or complex, should be noted.

Cork tissue is present in most bark powders, those alone from which it has been stripped being more or less completely destitute of it. It offers considerable resistance to the drug mill, and is usually found in small flattened portions that expose their surface view to the observer. The size of the cells, thickness of the walls, nature and colour of contents, may all afford useful confirmatory evidence.

The parenchymatous tissue of the barks is not, as a rule, characteristic except as regards the cell contents. These are mostly starch and calcium oxalate, and their presence as well as the forms they affect should not be neglected.

Secretory tissue has also here, as with the leaves, distinct diagnostic value. Large oil glands, and often oil cells also, are so destroyed by the grinding as to be difficult of detection. Elongated secretory cells, especially if filled with a secretion of characteristic colour, are, on the other hand, more easy to find.

General Scheme of Examination.—The following general scheme may be adopted for examining barks :

1. Preparation of transverse sections; examination of the tissues present, determination of their distribution; preparation of a diagrammatic sketch indicating these, and sketches of portions of the section on a scale large enough to include all details.
2. Preparation and examination of radial sections; sketches of important details.
3. Isolation of the tissues by maceration, either (a) with caustic potash, or (b) with potassium chlorate and nitric acid.
4. Examination of the powder, both coarse and fine.

Cascara Sagrada

Source.—The bark of *Rhamnus Purshiana*, D.C.

Preparation and Examination of Transverse Sections.—

Select a thin piece of bark, and cut from it several small pieces

about $\frac{1}{2}$ inch long and $\frac{1}{4}$ or $\frac{1}{8}$ inch wide. Soften them by exposing them to a moist atmosphere for twelve or twenty-four hours. Embed in pith, as already described, and cut transverse sections. Place these at once in alcohol to free them from colouring matter and air.

Transfer one or two of the thinnest to a drop of water on a slide, spread out with the needles, add a drop of glycerin, and examine. If the structure is not sufficiently distinct, mount another section in chloral hydrate.

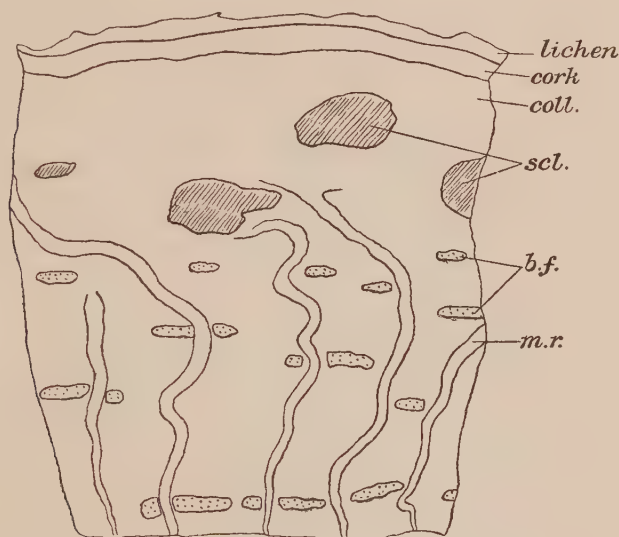


FIG. 84.—Cascara Bark, diagrammatic transverse section. *b.f.*, bast fibres; *coll.*, collenchymatous tissue; *m.r.*, medullary ray; *scl.*, sclerenchymatous cells.

On the outside a comparatively narrow layer of small cork cells can be seen, and recognised by their reddish-brown contents. Here and there the cork may be covered by a whitish coating of lichen, the structure of which may be passed over. Trace the medullary rays from the cambium towards the cork as far as possible. Prepare a diagrammatic sketch of the section in which the extent and relative positions of these tissues (but not the constituent cells) are shown (compare fig. 84). Stain one or two sections with phloroglucin and hydrochloric acid. Observe groups of cells which are stained deep red. Some of these are groups of sclerenchymatous cells,

others are groups of sclerenchymatous fibres (bast fibres); in the latter the individual cells are easily distinguished; they are small, rounded or polygonal, and almost completely filled with secondary thickening. Notice that the groups of bast fibres are narrow, tangentially elongated, and stretch from one medullary ray nearly, or quite, to the next; they are arranged in fairly regular tangential lines. Introduce the groups of sclerenchymatous cells and fibres into the sketch, outlining them only, but keeping the sizes and positions of the groups correct. Key the sketch with letters indicating the nature of each of the tissues. The cortex is usually not sharply delimited.

Next proceed to examine each of these tissues more in detail.

1. *Cork*.—This consists of several rows of narrow, flattened cells with thin walls and dark reddish-brown contents. The arrangement of the cells in regular radial rows indicates their formation from a phellogen. The walls of the cork cells are suberised (reactions with sulphuric acid and with Soudan red). The innermost row of cells is the phellogen (provided no phelloderm has formed).

2. *Phelloderm*.—This tissue is developed, at most, in small quantity only; the cells are distinguished from the phellogen cells by having thicker walls; from the cork cells by not being suberised, and from the cells of the cortex (usually) by their arrangement in radial rows.

3. *Cortex*.—The cells are larger than those of the phelloderm, and are not arranged in radial rows. Near the cork they may exhibit collenchymatous thickening of the wall and be tangentially elongated, but towards the bast ring they are usually rounded, thin-walled, with intercellular spaces. The pits are distinct; some of the cells have walls that are more or less evidently reticulately thickened.

While the outer limit of this tissue is easy to determine in the bark under examination, the inner limit is not. The endodermis, which in young stems sharply delimits the cortex, has long since disappeared, and the cortex passes insensibly into the bast ring. Since the former contains no medullary rays, it is quite certain that the tissue in which these can be traced is part of the bast ring. But the medullary rays commonly become indistinct near the outer limit of the bast ring, and this indication is therefore not a precise one. Here

and there (but by no means in all sections), not far from the cork, a group of flattened oval fibres with moderately thick walls may be found. These are pericyclic fibres, and indicate fairly accurately the limit of the cortex.

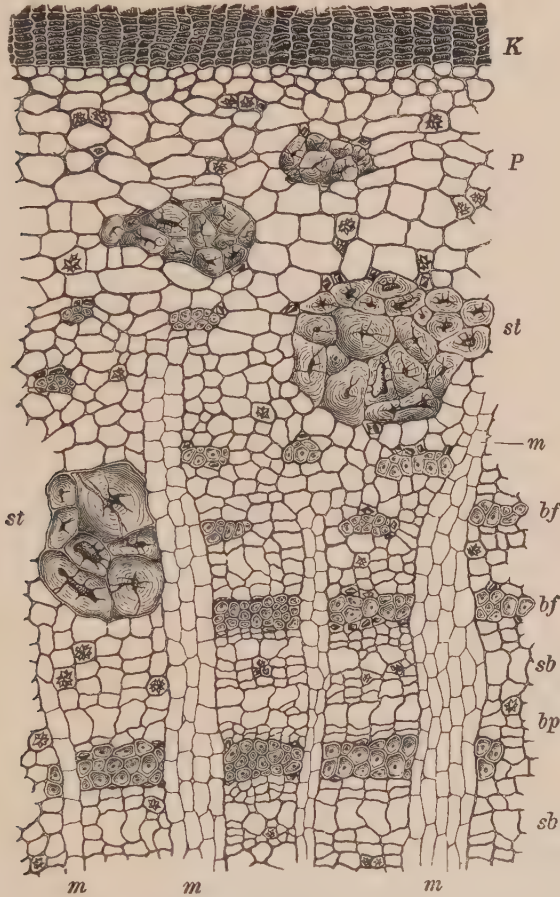


FIG. 85.—Cascara Bark, transverse section; *bf*, bast fibres; *bp*, bast parenchyma; *K*, cork; *m*, medullary rays; *P*, cortex; *sb*, sieve tubes; *st*, groups of sclerenchymatous cells. (Moeller.)

The cortex contains also groups of sclerenchymatous cells. These groups are usually irregularly rounded or tangentially elongated; the cells of which they consist vary very much in size, but agree in having very thick and conspicuously striated walls, through which branching pits run; the lumen is often

nearly filled up, and the cells when stained with phloroglucin and hydrochloric acid assume a deep pink colour.

The parenchymatous cells of the cortex contain chlorophyll and also calcium oxalate; the latter is usually in rosette crystals except near the sclerenchymatous cells, where prisms are commonly present. There is little or no starch to be found.

4. *Bast Ring*.—As already indicated, the tissue referred to under this name may contain the pericycle, the primary and secondary bast, and its external limit is very ill defined. In the bark under examination, if the groups of flattened, oval, pericyclic fibres cannot be identified, there remains no course open but to trace the medullary rays as far as can be done and to speak of the whole of this tissue as the bast ring. It usually consists almost entirely of secondary bast. The latter may be divided into medullary rays and bast rays. In cascara bark the former exhibit the usual characters; they are commonly from two to four cells wide, and contain a yellow substance, which, however, is easily soluble in alcohol.

The bast rays are important, and require very careful examination. Note in them occasional groups of sclerenchymatous cells such as have already been described. Note also the presence of narrow tangentially elongated groups of bast fibres. The individual fibres are polygonal or nearly rounded in outline (in transverse section), uniform in size, and so much thickened that the cavity is reduced to a point. In the phloroglucin preparation they are stained red, but not so strongly as the sclerenchymatous cells; they are, therefore, with the exception of the middle lamella, less strongly lignified. These fibres exhibit occasional pits which, however, are unbranched. The student should carefully compare groups of fibres with groups of sclerenchymatous cells. The two cell forms can usually be distinguished by the transverse section alone, but more definite information is afforded by a longitudinal section.

Notice in the parenchymatous cells that abut on the fibres small prisms of calcium oxalate.

The other tissues present in the bast rays of cascara bark are sieve tubes and bast parenchyma. Of these the former are the more important. They are a never-failing constituent of barks, and must therefore be carefully examined.

Stain a section with corallin soda (preparing a fresh solution by dissolving a trace of corallin in a little 25 per cent.

solution of sodium carbonate), mounting it in the reagent. Examine the part of the section near the cambium, and note masses in some of the cells that assume a brilliant pink colour. These are the masses of callus deposited on the sieve plates, and the cells in which they occur are sieve tubes. The latter are larger than the parenchymatous cells of the bast, and usually alternate with them in tangential bands (fig. 85, *sb*). Examine an unstained section; the sieve tubes can now be generally identified and the sieve plate detected. Sometimes this plate is transverse, but more often oblique; hence a portion only of the sieve plate is usually seen. In many no callus has been formed, and these do not stain with corallin; they may, however, be identified by their larger size and rather thicker walls, which are also less uniform than those of the bast parenchyma.

Examine a transverse section mounted direct in water (without, therefore, previous treatment with alcohol). Note the granular, dingy yellow contents of the parenchymatous cells of the cortex, bast, and medullary rays. Irrigate with solution of caustic potash; the yellow substance instantly dissolves, with production of a bright purple colouration.

Having thus thoroughly examined the section, the student should sketch small portions on a comparatively large scale; these should include

1. A portion of the cork, taking care to reproduce accurately the shape of the cells and thickness of the walls;
2. A portion of the bast ring, including a group of bast fibres, the sieve tubes and bast parenchyma, and the medullary ray on either side.
3. A group of sclerenchymatous cells.

Radial Sections.—Next cut radial sections and treat in the same way. Identify and examine the elements that have been seen in the transverse sections.

The cork cells present an appearance very similar to the transverse section, but the collenchymatous cells of the cortex have generally a rounded and smaller lumen; the latter cells are therefore tangentially elongated. The inner rows of cortical parenchyma have thinner walls, rounded section, and show intercellular spaces.

The groups of sclerenchymatous cells also closely resemble

those seen in transverse section. The bast fibres, however, are seen to be long prosenchymatous elements, with a very small lumen and few pits, the latter with difficulty visible in chloral hydrate. Each group is bordered by regular rows of small cells, each of which contains a prism of calcium oxalate.

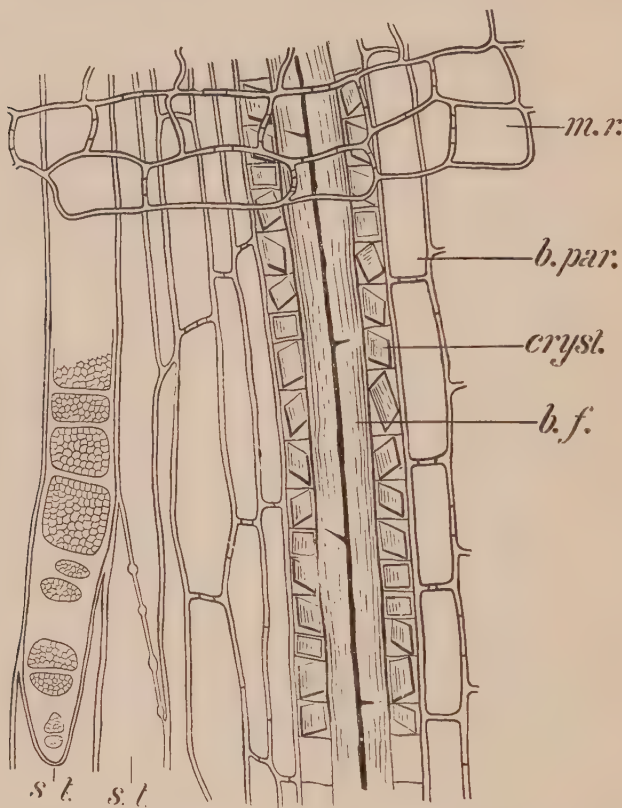


FIG. 86.—Cascara Bark, radial section. *b.f.*, bast fibre; *b.par.*, bast parenchyma; *cryst.*, crystal; *m.r.*, medullary ray; *s.t.*, sieve tubes. $\times 450$.

The medullary rays are also easily seen in radial sections as plates of radially elongated cells (compare Quassia Wood).

Next identify the sieve tubes, aiding identification, if necessary, with corallin-soda. They are long wide tubes, showing at intervals oblique sieve plates. They are easily distinguished from the parenchyma of the bast, the cells of which are smaller, shorter, and have thinner walls and square ends, but

which often have large pores that may be mistaken for small sieve plates. Note also the beaded appearance of the sieve plates in transverse section, and compare with the beaded appearance sometimes exhibited by the bast parenchyma in similar sections.

Isolation of the Elements by Maceration with Potash

Cut from a small piece of bark several longitudinal strips about $\frac{1}{16}$ inch wide. Cut these again transversely into pieces about $\frac{1}{8}$ inch long. Macerate several of these on a water-bath for five to fifteen minutes in a solution of potash containing 2 per cent. of caustic potash. Wash with distilled water. Transfer one to a slide, tease out the different tissues with the needles, and examine. Nearly all the parenchymatous cells contain a bright purplish colouring matter. The cork is easily identified; it consists of plates (surface view) of polygonal cells with brown contents. The bast fibres are very conspicuous by reason of their yellow colour and accompanying crystals of calcium oxalate. The sclerenchymatous cells are seen as rounded groups of yellow, thick-walled cells. The sieve tubes can also be identified (corallin-soda); they often remain connected with one another if the action of the alkali has not been too energetic.

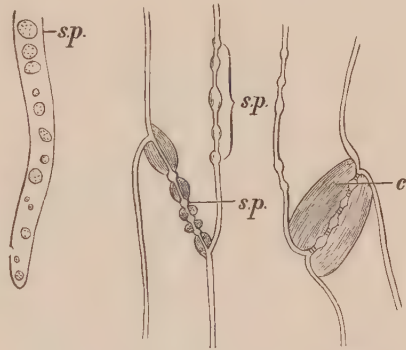


FIG. 87.—Sieve Tubes of Cascara Bark.
sp., sieve plates; *c*, callus. $\times 320$.

Sketch a sieve tube, with its sieve plate.

Examination of the Powder.—Reduce a little cascara bark to a coarse powder. Sift this first through a No. 20 and then through a No. 60 sieve, so as to separate it into coarse, medium, and fine powder. Examine the coarse fragments with the lens, and endeavour to pick out some with the needle or brush. Fix them on pith as directed for bearberry leaves, and cut from them sections, which can then be compared with the sections made from the drug itself. The medium powder

may be examined in the same way, and part of it may be reduced to a fine powder and treated as described below for that which passes through a No. 60 sieve.

Mount a little of the fine powder in water or dilute glycerin, and examine. Note the reddish-brown colour of the contents of the cork cells, and the yellowish colour of those of the parenchymatous cells; add a little solution of potash, and observe in the latter the change to purple.

To another portion of the powder, previously moistened with alcohol and allowed to nearly dry, add chloral hydrate. This reagent rapidly clears the tissues; with a little care most of the colouring matter can be washed out by irrigation. Observe in this preparation particularly the following details:

1. *Cork*.—The cells are polygonal, and have rather thin walls without intercellular spaces; they have reddish-brown contents.
2. *Sclerenchymatous Cells*.—These are in oval or rounded groups; the walls are very thick, striated, and traversed by branching pits; the details of the individual cells are often difficult to distinguish.
3. *Sclerenchymatous (Bast) Fibres*.—They are mostly in groups, usually conspicuous by their yellow colour and by the rows of crystals that accompany them; the shape of the fibres can generally be ascertained.
4. *Bast Parenchyma*.—Axially elongated parenchymatous cells, with large rounded pits; the walls in section often appear beaded.
5. *Sieve Tubes*.—These are easily identified by their size as well as by the sieve plates that they contain. They are often accompanied by bast parenchyma, which remains attached to them; they are particularly easy to find in a preparation stained with corallin-soda (see below).
6. *Medullary Rays*.—The cells occur in plates, and cross the bast parenchyma, with which they often remain associated, at right angles; the transverse walls are pitted.
7. *Cortical Parenchyma*.—Large rounded cells, often with intercellular spaces, and containing here and there rosette crystals of calcium oxalate.

With a little experience these various cells and tissues can be identified without further experiments; but the following

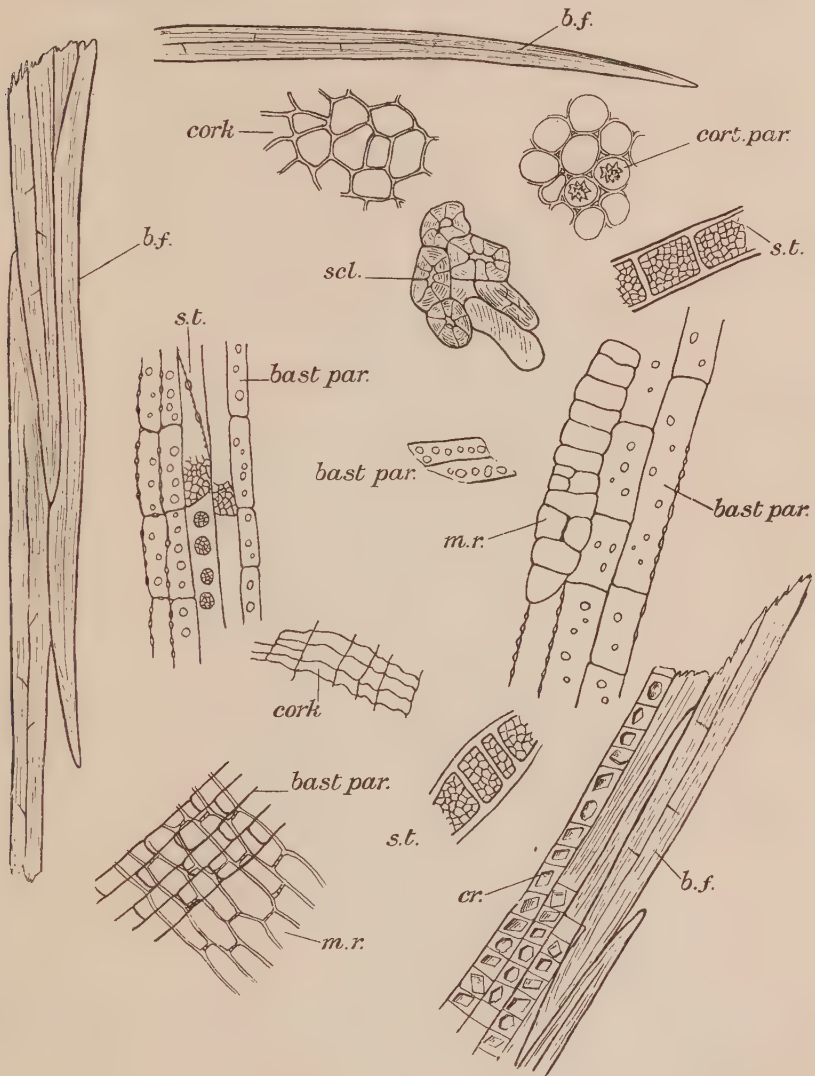


FIG. 88.—Powdered Cascara Bark. *b.f.*, fragments of bast fibres, with and without crystal cells adhering; *cork*, cork, in surface view and section; *m.r.*, medullary ray, in tangential and radial section; *scl.*, sclerenchymatous cells in group; *s.t.*, fragments of sieve tubes. $\times 220$.

preparations, made with a little of the powdered bark that has been freed from colouring matter, either by exhaustion with

chloral hydrate or by maceration for a few minutes with solution of chlorinated soda and washing, may be useful to the student.

1. Mount a little of the bleached powder in corallin-soda ; the callus plates stain pink ; by this means the sieve tubes can be identified ;
2. Stain a little with phloroglucin and hydrochloric acid ; the bast fibres and sclerenchymatous cells stain red ;
3. Warm a little with Soudan red, cool, and after a few minutes examine ; the cork cells stain reddish.

Aldbuckthorn Bark

Source.—The bark of *Rhamnus Frangula*, Linn.

Examination.—Treat this bark exactly as the preceding. Note the following differences.

1. Most of the cork cells contain a bright purplish colouring matter ;
2. There are no sclerenchymatous cells in the cortex or bast ring.
3. The colour of the contents of the parenchymatous cells is rather a bright yellow.

In the powder the same differences may easily be observed, and the powders of the two barks may readily be distinguished. In mixtures of the two powders cascara bark can be identified by its sclerenchymatous cells, and aldbuckthorn by the purple colour of the contents of the cork cells.

Witchhazel Bark

Source.—The bark of *Hamamelis virginiana*, Linn.

Preparation and Examination of Sections.—Select some pieces of the bark, if possible with cork attached ; cut small pieces, and soften in a damp atmosphere. Cut transverse sections, and treat them as directed for cascara bark.

The outside layer is cork ; the cells of which it is composed are rather large, and have thin walls ; they are nearly isodiametric, or at least not conspicuously flattened ; most of them are empty, but some contain a red brown amorphous substance. If the cork is well developed a layer of thin-walled cells

several rows wide may be found alternating with one or two rows of thick-walled pitted cells.

Within the cork there is usually a phelloderm consisting of several rows of cells. These may be identified as phelloderm

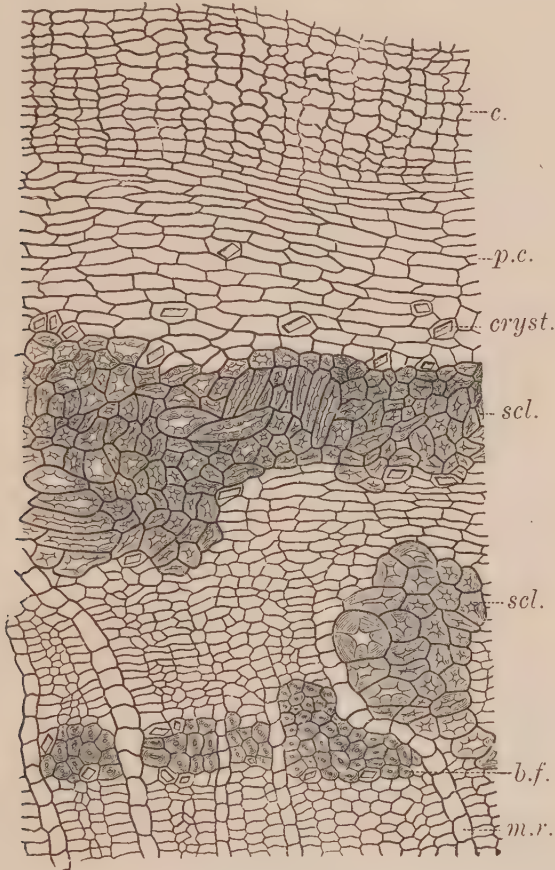


FIG. 89.—Witchhazel Bark, transverse section. *b.f.*, bast fibres; *c.*, cork; *cryst.*, prismatic crystals of calcium oxalate; *m.r.*, medullary ray; *p.c.*, primary cortex; *scl.*, sclerenchymatous cells. $\times 130$.

by the regular radial rows in which the cells are arranged, showing that they have originated from a line of merismatic cells.

Mount a section in water, remove the water with filter paper and drop on solution of chlorzinciodine; the phelloderm

cells colour bluish violet (cellulose reaction), while the cork cells colour yellow (suberin reaction).

The phelloderm passes into primary cortex ; the commencement of the latter tissue may in this case (but not always) be recognised by the position of the radial walls, which are no longer continuous with those of the phelloderm, but alternate with them. The inner rows of cells belonging to the primary cortex are often tangentially elongated.

This tissue is distinguished by containing large, well-formed prismatic crystals of calcium oxalate (the previous barks containing rosettes).

Passing through the primary cortex towards the cambium a complete or slightly interrupted ring of sclerenchymatous cells is reached, some of which are very large ($100\ \mu$ long or even more), many of medium size (40 to $60\ \mu$), some very small (10 to $20\ \mu$). These have been developed from the cells of the pericycle, and they do not belong, therefore, to the primary cortex.

The tissue within the sclerenchymatous ring consists of primary and secondary bast. In this tissue there occur groups of sclerenchymatous cells of varying dimensions ; they resemble those of the sclerenchymatous ring.

The medullary rays are one cell wide.

The bast fibres are conspicuous by reason of their number ; they are strongly thickened, arranged in tangentially elongated groups, which stretch from one medullary ray to another, and are accompanied by crystal cells.

The sieve tubes and bast parenchyma which compose the remainder of the bast ray resemble those of cascara, but the sieve tubes are smaller, less easy to distinguish, and do not always stain with corallin ; they are therefore rather more difficult to identify.

Having thus investigated the structure of the bark as exhibited by transverse sections, mount a section direct in water ; note the general absence of colour ; some only of the cork cells, of the sclerenchymatous cells, and of the parenchyma contain an amorphous reddish-brown substance. Many appear dull pale greyish-brown in colour.

Irrigate with solution of potash ; there is no striking change of colour.

Cut several transverse sections from bark that has been

softened by exposing it to a moist atmosphere. Transfer one direct to a drop of dilute solution of ferric chloride ; the whole of the parenchymatous tissue, including the medullary rays, especially the parenchyma of the secondary bast, is coloured deep bluish black (reaction for tannin) ; the cells of the cortex and of the cork are less strongly coloured ; the sclerenchymatous cells and bast fibres remain quite colourless.

This reaction indicates that in the drug the tannin is contained in the parenchymatous cells ; but as this substance easily passes from the cells in which it was originally contained into the surrounding tissue, it does not necessarily follow that it was originally present in all these cells.

A number of other substances give a dark bluish or greenish colouration with ferric salts,¹ and the reaction does not, therefore, necessarily indicate the presence of tannin.

Mount another section direct in Braemer's reagent ; the parenchymatous cells assume a yellow to dark reddish-brown colouration. This reaction is more particularly distinctive of tannin, and has the advantage of producing a precipitate which cannot diffuse from the cells in which it is produced.

Next examine tangential and radial sections, and identify in them the elements that have been observed in the transverse section. The sieve tubes are in these sections, as in the transverse, not so conspicuous as they were in cascara, but they may be found by careful searching.

For the isolation of the bundles of bast fibres employ digestion with solution of potash, and for the separation of the bast fibres from one another maceration with potassium chlorate and nitric acid.

Examination of the Powder. — Proceed next to examine the powder as follows :

1. Mount a little in water or dilute glycerin. There is no conspicuous colour ; the bast fibres and sclerenchymatous cells are nearly colourless, but some of the latter contain a red brown colouring matter, as do also many of the parenchymatous cells and some of the cork cells.

2. Irrigate the preparation gently with solution of potash ; there is no striking change of colour, but the bast fibres and sclerenchymatous cells acquire a yellowish tinge.

3. Mount a little in chloral hydrate (after alcohol) : in this

¹ A list of these has been given by Braemer, *Les Tannoïdes*, p. 125 (1890).

preparation the sclerenchymatous cells are conspicuous by reason of their number. Some are isolated, but the majority

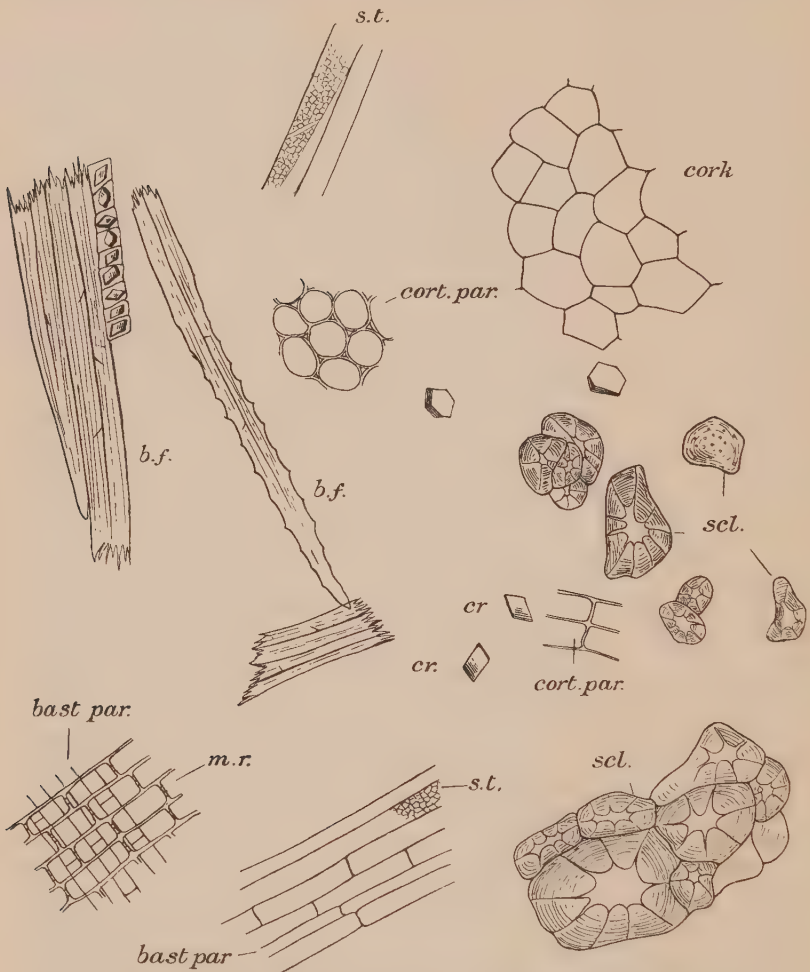


FIG. 90.—Powdered Witchhazel Bark. *b.f.*, fragments of bast fibres, with and without crystal cells adhering; *cork*, cork cells in surface view; *cr.*, crystals of calcium oxalate; *cort. par.*, cortical parenchyma, in transverse and longitudinal section; *m.r.*, medullary ray, in radial section; *scl.*, sclerenchymatous cells, isolated and grouped; *s.t.*, sieve tubes, more or less broken. $\times 210$.

are in groups of varying size; the cells themselves also exhibit great diversity in size, shape, and the extent to which they have been thickened.

Bast fibres are also conspicuous ; they are nearly colourless and accompanied by an abundance of calcium oxalate crystals ; the cavities are very narrow.

Fragments of parenchymatous cells are abundant ; among these comparatively large, well-defined prismatic crystals of calcium oxalate can be detected, but no rosettes. Beaded parenchymatous walls are not numerous, and comparatively few have large pits.

The medullary ray cells are easily identified ; they exhibit no striking features.

The sieve tubes are inconspicuous ; they seldom attain the size that is common in cascara bark (see fig. 90).

4. Treat some of the powder with potassium chlorate and nitric acid as directed for quassia wood ; the sclerenchymatous cells and the bast fibres may be isolated, and their exact size and shape determined (but care must be taken not to unduly prolong the action of the oxidising mixture).

Cinnamon Bark

Source.—The bark of *Cinnamomum zeylanicum*, Breyn., freed from the epidermis and most of the cortex.

Preparation and Examination.—From a stick of cinnamon separate one or two of the outer pieces of the thin bark ; soak them for twelve hours in water, or expose them for two days to a moist atmosphere ; fix three or four together between pith ; cut them all together, and transfer the sections to a small dish. Then fix the pieces so as to cut radial sections, and transfer these to another dish.

Examine a transverse section in chloral hydrate. Most of the cells of the bark are coloured dark brown.

Decolourise some sections by placing them in solution of chlorinated soda for a few minutes ; as soon as they are decolourised transfer them to water. Mount one in a solution of chloral hydrate, and examine.

The outermost tissue is (with the exception of fragmentary parenchymatous cells) a band of sclerenchyma within which is the narrow bast ring traversed by medullary rays. There is no epidermis or cork, and traces only of primary cortex.

Examine the sclerenchymatous ring ; the cells of which it is composed are mostly tangentially elongated (in transverse

section), but some are nearly round ; they may attain 150μ or more in length. They have the characters that such cells usually possess, but are distinguished by the fact that the inner walls of many of them are thicker than the outer. Search also in this ring for groups of fibres that may easily be recognised by their small size, complete thickening, and absence of pits ;

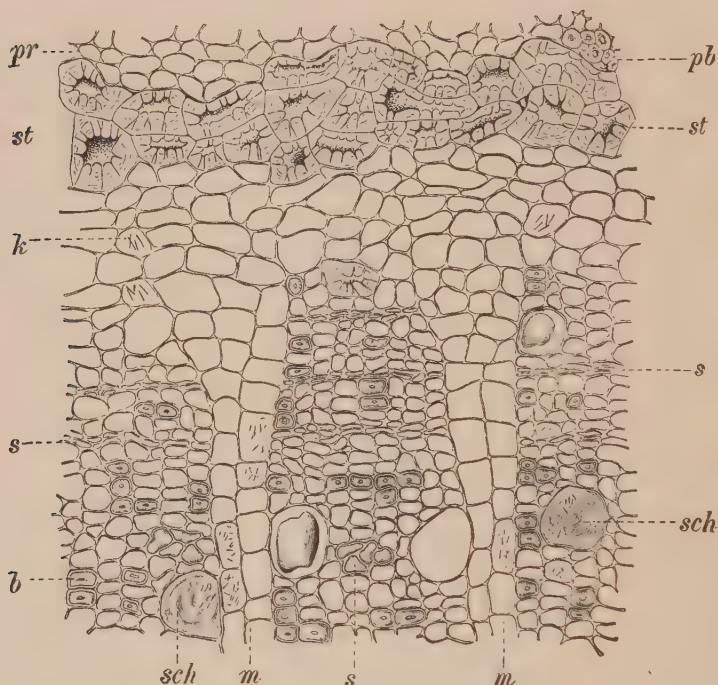


FIG. 91.—Cinnamon Bark, transverse section. *b*, bast fibres; *k*, crystals of calcium oxalate; *m*, medullary rays; *pb*, primary bast fibres (pericyclic fibres); *pr*, cortical parenchyma; *s*, sieve tubes; *sch*, secretion cells; *st*, sclerenchymatous cells, forming an uninterrupted ring. $\times 160$. (Moeller.)

they are usually on the outer margin of the ring. These are groups of pericyclic fibres (primary bast fibres).

Examine the bast ring. The parenchymatous tissue abutting upon the sclerenchyma consists of tangentially elongated cells. The medullary rays are mostly two cells wide, and the cells are nearly isodiametric. The bast rays contain conspicuous bast fibres, rounded or four-sided in section ; they are isolated or in small, often tangentially

arranged, groups of three or four. In the bast rays observe also the secretion cells. They are often empty; they may be distinguished from the bast parenchyma by their larger size, and may be made more conspicuous by warming a section in Soudan red, by which the walls are coloured red.

The sieve tubes can be recognised by the characters of the walls; towards the sclerenchymatous ring they often collapse into strands, in which the cell cavities are scarcely visible.

Mount a bleached section in corallin soda—many of the sieve tubes will show rounded masses of callus stained pink.

Notice in some of the cells of the medullary rays and bast parenchyma numerous, minute, prismatic crystals; they are calcium oxalate, and will appear brilliantly illuminated when examined by polarised light. Among other cell contents to be examined note the starch grains.

Make a diagrammatic sketch of the transverse section and enlarged sketches of a portion of the sclerenchymatous ring and of the secondary bast.

Treat radial sections as directed for transverse.

The secretion cells are easily seen; they are axially elongated; some are empty, but some contain yellowish volatile oil (or resin), or a mixture of this with mucilage, or mucilage alone.

The sieve plates are small, transversely or slightly obliquely situated, and very numerous. The sclerenchymatous cells are nearly isodiametric; the parenchymatous cells next them are rather thick-walled and isodiametric, or slightly axially elon-

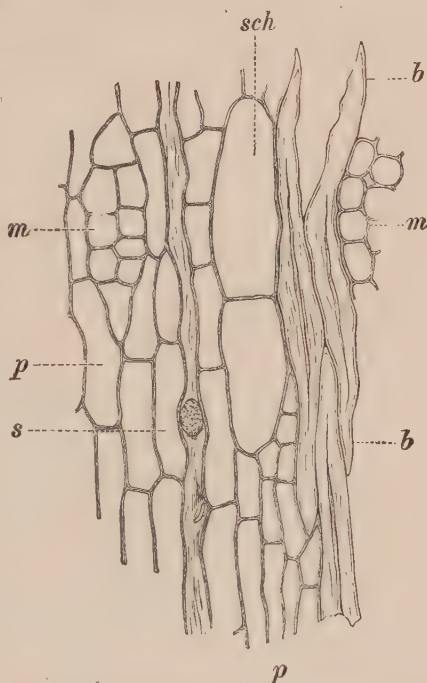


FIG. 92. Cinnamon Bark, tangential section. *b*, bast fibres; *m*, medullary ray; *p*, bast parenchyma; *s*, sieve tube; *sch*, secretion cells. $\times 160$. (Moeller.)

gated. The bast parenchyma cells have thinner walls ; they are strongly axially elongated, and exhibit large circular pits. The cells of the medullary rays are larger, squarer, sometimes but not always radially elongated, and have very thin walls ; they can therefore be easily identified.

Treatment by Maceration, &c.—Digest a few fragments of the bark in 5 per cent. solution of potash on a water-bath for fifteen minutes ; wash with distilled water, and tease out with the needles on a slide. The bast fibres can by this means be easily isolated, since they often occur singly. Examine and sketch one or two, preserving accurately the relative length and thickness.

In this preparation numerous secretion cells containing droplets of yellowish oil or brown resin will be found. Sketch one or two.

From a small portion wash out the potash with distilled water ; mount in corallin soda ; the callus plates stain pink, but not as well as in the section bleached with chlorinated soda.

Macerate a few pieces of the bark with potassium chlorate and nitric acid, as directed for quassia wood ; isolate, and examine the sclerenchymatous cells ; observe the frequent one-sided thickening. Sketch three or four cells.

Examination of the Powder.—Reduce some picked cinnamon to powder, and sift it through a No. 60 or No. 80 sieve.

Mount a little in water or dilute glycerin ; examine the starch. The grains are small, often two, three, or four together. Sketch a few, preserving, again, accurately the relative size.

Mix 0·2 gramme of the powder with 10 c.c. of solution of chlorinated soda. Shake occasionally until the brown colour is changed to yellowish. Separate the powder by allowing it to settle or by the use of the centrifuge. Wash once with distilled water, and separate again. Pour off the supernatant liquid, and treat the residue as follows :

- (1) Mix a little of the deposit with glycerin and examine. Observe in this preparation the bast fibres, starch, calcium oxalate, and cellular elements. The sieve tubes and secretion cells are not easily seen.

- (2) Transfer a little to a slide, remove most of the water with filter paper, add a little Soudan red in glycerin, and warm gently to the boiling-point; let the slide stand for five or ten minutes, and examine. The secretion cells will be readily distinguished by the red colour which their suberised walls will have assumed. Others of the cell walls also frequently take a faint colour, but not nearly so deep as those of the secretion cells. Droplets of oil in the preparation are also stained deep red.
- (3) Stain a little with corallin-soda; the callus plates stain pink, rendering the sieve tubes easy of identification. Compare them with the section.
- (4) Warm a little of the deposit with water or chloral hydrate. The starch will be gelatinised, and the minute, prismatic crystals of calcium oxalate can easily be seen, both in the cells and scattered over the field. By polarising they can be made particularly conspicuous. In this preparation the characteristic pits on the walls of the parenchymatous cells can also be seen.
- (5) From a little of the deposit remove the water and add a drop of strong hydrochloric acid; cover, and allow it to stand five or ten minutes. Then remove the acid, and add a drop of glycerin. The bast fibres and sclerenchymatous cells are now particularly well seen, and can be minutely examined.

By means of these preparations the following characteristic elements of cinnamon bark will have been detected in the powder: bast fibres, sclerenchymatous cells, secretion cells, starch, calcium oxalate, sieve tubes, cells of medullary rays and parenchymatous cells with characteristic pits.

Coarse powder may be separated by sifting as described for senna, the fine powder being examined as above, while from the coarsest fragments sections should be prepared. Part also of the coarse portion should be reduced to fine powder, and compared with that separated by sifting.

Cassia Bark

Source.—The bark of *Cinnamomum Cassia*, Blume.

Examination.—Cassia bark may be examined in the same way as cinnamon bark. The transverse section often exhibits a layer of cork in which rows of cells with thickened walls alternate with cells with thin walls.

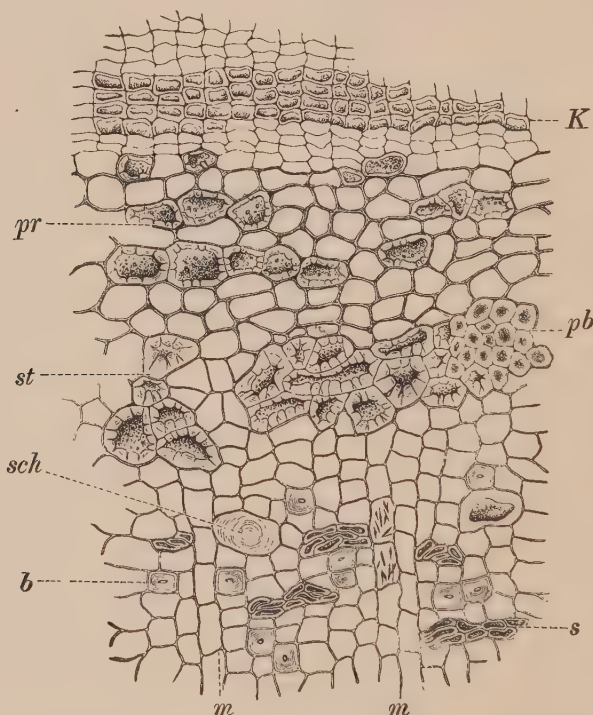


FIG. 93.—Cassia Bark, transverse section. *b*, bast fibres; *K*, sclerenchymatous cork cells; *m*, medullary rays; *pb*, primary bast fibres (pericyclic fibres); *pr*, cortical parenchyma, with sclerenchymatous cells; *s*, sieve tubes; *sch*, secretion cell; *st*, sclerenchymatous cells, forming an interrupted ring. $\times 160$. (Moeller.)

The distinction of powdered cinnamon from powdered cassia is not difficult if fine grades of cinnamon are compared with typical samples of cassia. In the latter the starch grains are rather larger and the bast fibres stouter. But these differ-

ences, which are themselves not great, diminish as one compares the lower grades of genuine cinnamon with the finest grades of cassia. In such cases the powders are extremely difficult to distinguish.

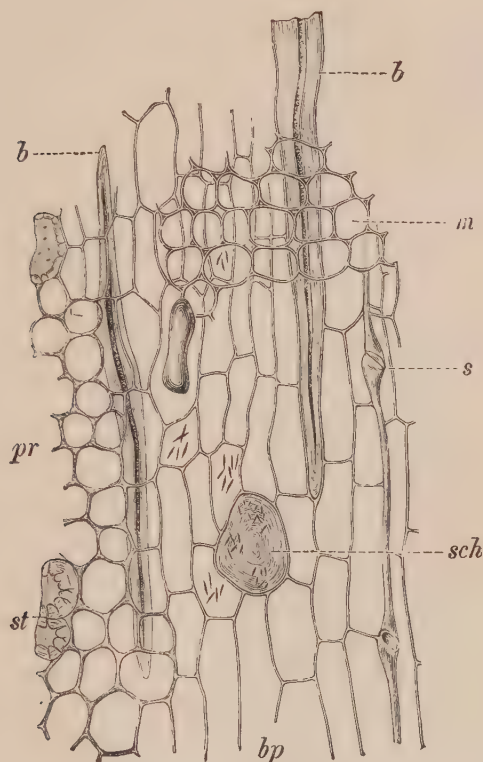


FIG. 94.—Cassia Bark, radial section. *b*, bast fibres; *bp*, bast parenchyma; *m*, medullary ray; *pr*, cortical parenchyma; *s*, sieve tube; *sch*, secretion cell; *st*, sclerenchymatous cells. $\times 160$. (Moeller.)

Red Cinchona Bark

The bark of *Cinchona succirubra*, Pav.

Preparation and Examination of Sections.—For examination choose a rather thin, young, cultivated bark in quills; prepare it by soaking in water for twelve hours; cut transverse sections, and treat them in the usual way. Mount one in water. The section does not expand well in water; add a drop

of solution of potash; the colour deepens, and the section expands. Solution of caustic potash is frequently preferable to chloral hydrate, especially for hard barks and for such as contain much colouring matter resulting from the decomposition of tannin.

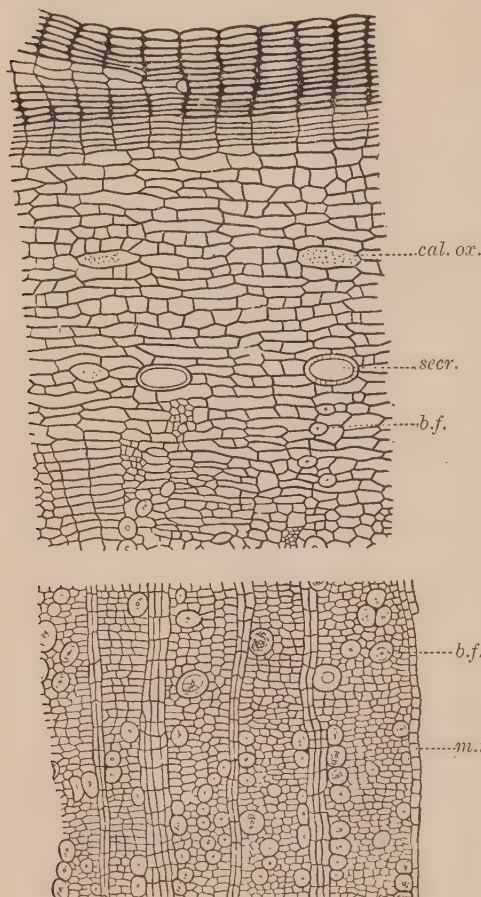


FIG. 95. - *Cinchona Succirubra*, transverse section of bark. *cal. ox.*, sandy crystals of calcium oxalate; *b.f.*, bast fibres; *m.r.*, medullary rays; *secre.*, secretion tubes. (After Tschirch.)

Observe the cork cells; they are very narrow, flattened, and contain a deep, reddish-brown amorphous substance, even after treatment with caustic potash. Next to the cork there is often a phelloderm of varying thickness; the cells are very much less coloured than those of the cork or primary cortex.

The primary cortex in young barks is often of considerable width; its inner limit is not very well defined, but on examination large isolated, usually empty, cells may be discerned near the commencement of the bast rays; they form a diffuse ring near the junction of the primary cortex with the bast ring. When young they contain a substance of gum-resinous nature, but in commercial barks they are often empty.

Longitudinal sections show these cells to be strongly axially elongated.

The walls of the cells of the primary cortex are encrusted with a reddish-brown amorphous substance and are collenchy-

matous at the angles. Some of them are filled with sandy crystals of calcium oxalate. The medullary rays are one to three cells wide, and exhibit no striking features. The bast fibres, on the other hand, are very conspicuous by reason of their size. They are isolated, and arranged in irregular radial rows. Many measure from 40 to 60 μ , some even 90 μ in diameter. The lumen is often reduced to a point; the wall is very distinctly striated, and traversed by rather numerous pores. These bast fibres are not accompanied by crystals.

The remainder of the bast rays is made up of bast parenchyma and sieve tubes, but the colour is so dark that but few details can be seen.

Remove the colour by bleaching the sections with chlorinated soda. Wash them once or twice in distilled water, and mount one in water.

The walls of all the cells can now be much more distinctly seen; those of the cortical parenchyma often exhibit large pores, but in the bast rays even now the sieve tubes can with difficulty be distinguished from the bast parenchyma.

Stain a bleached section with corallin-soda: the callus plates stain, and the sieve tubes can thus be identified.

Treat radial sections in the same way. Observe particularly the bast fibres; they are spindle-shaped, the lumen is narrow and the pits widen towards the lumen. The sieve tubes are small. The principal sieve plates are placed transversely or nearly so, but there are very small ones in vertical rows on the tangential walls.

Treatment by Maceration.—Digest some pieces of cinchona bark for about fifteen to thirty minutes in 3 per cent. solution of potash, and tease out.

The bast fibres are yellow; they are completely isolated, and their shape can be well seen. Observe numerous parenchymatous cells filled with prismatic crystals. These crystals are the precipitated alkaloids, and dissolve readily in dilute acid.

Stain the callus plates of the sieve tubes with corallin-soda, bleaching first with chlorinated soda as directed for cinnamon bark, if necessary.

Examination of the Powder.—Examine powdered red cinchona bark as follows:

- (1) In water. The bast fibres are mostly isolated; some are intact, some broken, but even small fragments are

easily recognised by their large size and characteristic pits; their colour is yellowish to yellowish-brown.

There are also very numerous fragments of yellowish to deep reddish-brown parenchymatous tissue.

- (2) In chloral. Warm in the water-bath for a few minutes, or allow it to stand several hours. The bast fibres become very distinct; the parenchymatous cells lose much of their colour, and can be identified. The cork is composed of polygonal cells, the colour of which is usually very dark brown.
- (3) After bleaching with chlorinated soda and staining with corallin-soda, for the detection of the sieve tubes.

The diagnostic characters of red cinchona bark are :

- (a) The remarkable bast fibres, with their characteristic pits;
- (b) Absence of other sclerenchymatous elements.
- (c) The dark reddish-brown colour of the parenchyma.

Identification of an Unknown Powder as that of a Bark

In the examination of a powder of unknown origin it may be necessary to identify it as that of a powdered bark. There is only one characteristic element that is present in all bark powders, and upon which therefore in this respect particular stress must be laid; this is the sieve tube. Its presence necessarily indicates the presence of bast tissue, and as barks are composed more or less largely of bast, the presence of numerous and comparatively large sieve tubes is strong presumptive evidence that the powder is derived from a bark. Cork tissue is also present in most powdered barks, but not necessarily, since it is sometimes removed during the preparation for the market. Nevertheless the occurrence of much cork tissue is strong confirmatory evidence, as is also that of bast fibres and sclerenchymatous cells.

All these elements may, however, be present in the bark that is attached to and constitutes a portion of such organs as roots, rhizomes, &c. It is certainly true that sclerenchymatous cells and fibres are not usually present in roots or rhizomes, but to this there are many exceptions.

A powdered bark should, be free from vessels, but fragments of wood may occasionally be found adhering to barks and constitute a source of contamination. Much vascular tissue would therefore indicate the presence of wood. Chlorophyll and the tissue in which it is chiefly found (palisade and spongy parenchyma) should also be absent, as well as epidermis; the presence of these would indicate admixture of leaf powder. Aleurone grains and fixed oil, characteristic reserve materials of seeds, should also be absent.

Having determined that the powder is derived from a bark and is free from contamination with powder derived from other organs, the endeavour may be made to establish its identity.

As is the case with leaves, this is at present a matter of considerable difficulty and requires great experience. Much information may be obtained from the published descriptions of the anatomy of the more important and more common barks.

SECTION IX

SEEDS

INTRODUCTION

THE student is strongly recommended to study carefully in his text-book of botany the structure of the seed ; the object of the following brief account is to direct his attention to those parts of the seed which usually afford valuable diagnostic characters.

The fully developed seed consists commonly of two seed coats, an outer and an inner, enclosing a kernel. Sometimes, however, only one seed coat is present, while occasionally there are three, the third being in the shape of an arillus or arillode. Now and then a caruncle is attached to the seed.

The kernel of the seed may consist simply of the embryo, or of the embryo accompanied by either endosperm or perisperm, or both.

In those seeds in which the kernel consists of the embryo only, in which, therefore, the tissues of the nucellus and embryo sac have not developed into perisperm and endosperm respectively, the remains of the younger stages of these two tissues are usually to be found, but the contents of their cells having been utilised by the embryo for its development, the empty cells become pressed together, and ultimately form delicate hyaline membranes, which exhibit perceptible structure in surface view only, and not always even then. Difference of opinion exists as to whether these membranous layers are to be reckoned as part of the seed coat or part of the kernel. Seeing that they afford an additional, if only very slight, protection to the embryo, I propose to include them with the seed coats, which therefore will embrace all the tissues of the seed exterior to the kernel.

The following parts may therefore be present in a seed, and each of them may furnish valuable diagnostic characters :

- | | |
|--------------------|--------------|
| 1. Caruncle | 5. Perisperm |
| 2. Arillus | 6. Endosperm |
| 3. Outer seed coat | 7. Embryo |
| 4. Inner seed coat | |

The seed coats exhibit an infinite variety of structure, but the following tissues may be mentioned as of frequent occurrence.

1. *Mucilaginous Layer*.—Many seeds, and especially dicotyledonous seeds, contain a layer of cells in which a quantity of mucilage has been secreted. As this mucilage is often destined to attach the seeds to the soil upon which they fall, it is usually the epidermal layer that is mucilaginous. Seeds that are thus provided with a mucilaginous epidermis surround themselves with a layer of mucilage when they are soaked in water, as mustard seed, linseed, and quince seed do.

2. *Sclerenchymatous Layer*.—Very frequently one or more rows of cells in the seed coats develop into a layer of sclerenchymatous cells or fibres, the object of which is to supply the seed coat with the necessary firmness and resisting power. This layer is often developed from the inner epidermis of the outer integument, though this is by no means necessarily the case (mustard, linseed) ; sometimes it is the outer epidermis of the same integument (henbane).

3. *Pigment Layer*.—The particular colour of the seed coat is often due to colouring matter deposited in a single (or occasionally multiple) layer of cells, as, for instance, in linseed and black mustard seed.

In other cases certain layers of the seed coats develop in particular ways ; thus in cardamom seeds, in addition to a sclerenchymatous layer, there is a single layer of large rectangular cells in each of which volatile oil is secreted. In *nux vomica* the epidermal cells of the outer integument develop into remarkable hairs, while all the remaining layers become obliterated ; in *areca* seeds sclerenchymatous cells of varying shapes are developed, together with much tannin, and so on.

The tissues derived from the outer and inner integuments of the ovule may be followed by obliterated layers derived from the nucellus or from the embryo sac, as well as by the proteid

layer. The latter is usually the epidermis of the endosperm, and is generally well preserved, the cells being moderately thick-walled and filled with proteid matter and oil. The obliterated layers of the nucellus and embryo sac often form hyaline membranes in which little structure is discernible, at least in transverse sections.

The kernel of the seed may consist of the embryo alone, or of the embryo and endosperm, or of the embryo, endosperm, and perisperm.

In structure the perisperm and endosperm are usually very similar. The cells of which they consist may have very thin walls, in which case the contents form the reserve material, or the walls may be thickened, sometimes to such an extent as almost to obliterate the cavity, the thickening being reserve cellulose or mucilage, or some modified form of cellulose (nux vomica).

In seeds that contain no endosperm the embryo itself usually fills with reserve material.

Very important for the present purpose is the determination of the thickness and nature of the cell walls of these tissues, as well as the character of the pits and the nature of the reserve material.

The infinite variety exhibited by the layers of the seed coats, both as regards the cells of which they are composed and the contents of those cells, renders the seeds very interesting to examine, and often easy to identify, in either the entire or the powdered state. Diagnostic characters are to be looked for particularly in the following cells and cell contents :

- (a) The epidermis of the seed coat ;
- (b) The sclerenchymatous layer ;
- (c) The pigment layer ;
- (d) The cell walls of the kernel ;
- (e) The reserve material of the kernel.

In addition to these, valuable diagnostic characters may be found occasionally in others of the layers present.

Aleurone Grains

In a preceding chapter the examination of starch grains was dealt with, and it was pointed out that they constitute a

most important means of identifying certain foods and drugs by reason of the varying characters that they exhibit. These characters are fairly constant for the starch grains of one and the same drug, but they vary in the grains of different drugs. Moreover, it is obvious that the presence of starch grains in a powdered drug which normally contains no starch indicates either substitution or adulteration, or possibly, as in the case of seeds, the substitution of the unripe for the ripe organ.

Starch is one of the commonest of the reserve materials of seeds. It is accompanied by proteid matter, which usually takes the form of minute rounded grains. In the pea, for example, the interspaces between the starch grains are packed with these minute grains of proteid matter embedded in a

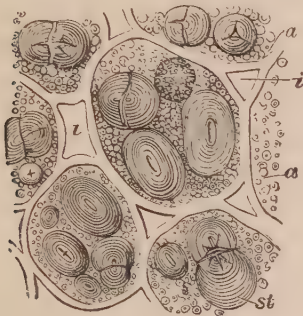


FIG. 96.—Section of the Cotyledon of Pea. *a, a*, aleurone grains; *i, i*, intercellular spaces; *st*, starch. Magnified. (After Sachs.)

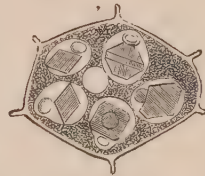


FIG. 97.—Cell from the Endosperm of Castor Seed, showing large transparent aleurone grains with globoids and crystalloids embedded in them. (After Sachs.)

granular ground substance. They can be readily seen if a very thin section from a dry pea be examined in glycerin to which a little solution of iodine in potassium iodide has been added, as under such conditions they acquire a yellowish brown colour (fig. 96). To these grains the name of *aleurone grains* or *proteid grains* has been given.

In many seeds, especially in such as contain oil or fat as the chief reserve material (in the place of starch), the aleurone grains attain a much larger size than they do in starchy seeds; they then occur immersed in the oil or oily plasma that fills the cells (fig. 97).

Like starch grains, the aleurone grains of one and the same drug exhibit a remarkable constancy in their characters, but they often differ in size, shape, or composition from the

aleurone grains of other drugs. Here, then, is a means by which it is possible within certain limits to distinguish one seed from another. This becomes especially important when the seed coats, in which the most valuable diagnostic characters reside, have been removed, and the chief means of identification thus destroyed. Such is the case with the flour prepared from decorticated seeds, or the very finely sifted flour from ordinary seeds, for in both cases but very few fragments of the seed coats are to be found, and the cells of the endosperm or cotyledons seldom exhibit any marked differences in the cell walls or cell contents, excepting the aleurone grains. Not only is this the case, but, since aleurone grains are found in ripe seeds only, it follows that their presence in a powder indicates the presence of a powdered seed or part of a seed.

It becomes very important, therefore, for the microscopist to study especially (a) the means by which aleurone grains may be recognised, and (b) the means by which the aleurone grains of one seed may be distinguished from those of another. For this purpose seeds containing fixed oil are preferable to those that contain starch, as their aleurone grains are larger and better developed.

The student should now make himself familiar with the principal characters and properties of these remarkable bodies.

The following constituent parts have been observed in aleurone grains—viz. *ground substance*, *crystalloid*, *globoid*, and *calcium oxalate crystals*. It is, however, comparatively rare to find all of them in one and the same grain.

Ground Substance.—The ground substance in which the crystalloid, globoid, &c., are embedded is quite amorphous and usually finely granular in appearance. It is generally readily soluble in water, or if not soluble in this medium it is always more or less vigorously attacked by it. Hence water is not a suitable medium in which to examine aleurone grains. Fixed oils and glycerin, on the other hand, have no appreciable action upon it. Prolonged (eight days) maceration in alcohol renders the ground substance insoluble in water; therefore, the characters of the aleurone grains cannot be accurately determined in seeds or sections that have been subjected to such treatment, but a short maceration (twelve hours) in absolute alcohol is advantageous, inasmuch as it makes the grains more resistant to water, and dissolves the fixed oil, which usually accompanies

them, so that the properties of the latter can be more easily studied.

In very dilute (0·3 per cent.) caustic potash the ground substance dissolves in all cases rapidly and completely. Lime water and dilute alkalis in general also dissolve it. It is also soluble in solutions of sodium chloride (1 to 10 per cent.), magnesium sulphate (1 to 20 per cent.), and disodium hydrogen phosphate (saturated solution).

Crystalloids.—The crystalloids consist, like the ground substance, of proteid matter, which, however, as the name indicates, has assumed a crystalline form; according to Schimper,¹ they belong either to the regular or to the hexagonal system. They differ from true crystals in the inconstancy shown by their angles and in the fact that under certain conditions they swell. They may be so large as to constitute by far the bulk of the grain, or they may be embedded in a larger amount of ground substance.

They are less soluble than the ground substance. They are seldom soluble in water, or in saturated solution of disodium hydrogen phosphate, but always dissolve in diluted solution of potash.

Both ground substance and crystalloid are coloured brown by iodine solution, yellow by saturated solution of picric acid, and red by Millon's reagent.

Globoids.—These bodies, which occur in many aleurone grains, are usually rounded or ovoid in shape. They consist, according to Pfeffer, of magnesium and calcium combined with phosphoric acid and with an undetermined organic acid. They are insoluble in water and in dilute caustic potash, but are soluble in dilute acids, in saturated solution of disodium hydrogen phosphate, and in solutions of various other salts. They do not colour with iodine or with picric acid, and the latter reagent may, under certain circumstances, dissolve them. They vary very much in size ($0\cdot5\ \mu$ to $10\ \mu$), but are generally small ($1\ \mu$ to $3\ \mu$). In those aleurone grains in which one or more crystalloids are accompanied by a globoid the latter is commonly situated at the narrower end of the grain (which is usually ovoid).

Calcium Oxalate.—This substance occurs most frequently in the shape of small rosettes, sometimes, but comparatively

¹ Zeitschr. f. Krystallographie, 1880.

seldom, in single crystals, or in groups of a few acicular crystals, It is easily identified by the usual microchemical tests.

Aleurone grains range in size from 1 to 55 μ in diameter and exhibit a great diversity of shape. Many are rounded ; this is

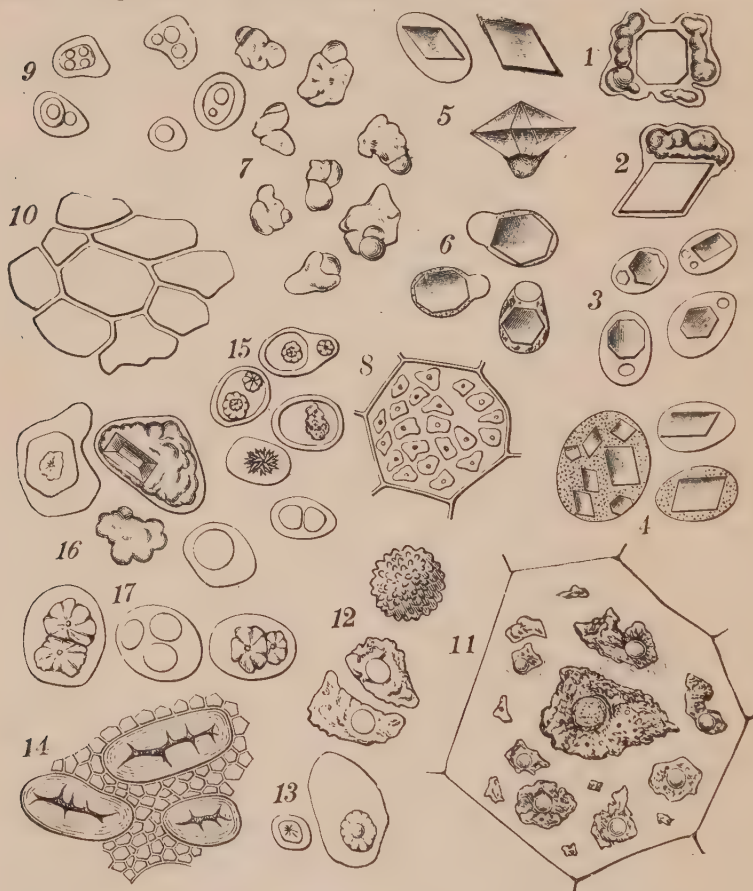


FIG. 98.—Aleurone Grains of various Seeds. 1 and 2, *Bertholletia excelsa*; 3, *Ricinus communis* (after treatment with water); 4, *Elaeis guineensis*; 5, *Myristica fragrans*; 6, *Cannabis sativa*; 7, *Datura Stramonium*; 8, *Gossypium*, sp.; 9 and 10, *Cydonia vulgaris*; 11, 12, 13, *Amygdalus communis*; 14, *Phaseolus vulgaris* (with starch grains); 15, *Coriandrum sativum*; 17, *Feniculum*, sp. (Tschirch.)

especially the case with the very small ones ; frequently they are ovoid or polygonal, or irregularly angular. Sometimes each cell contains a large grain accompanied by a number of small ones, or it may contain several of varying size, or a few or many of tolerably uniform size.

In their composition they also exhibit great variety. Very often the grain contains one globoid and from one to three crystalloids embedded in a rather scanty ground substance. Others contain numerous minute globoids in a large quantity of ground substance, and so on.

The following experiments will serve to introduce the student to a suitable method for recognising and examining these bodies.

Take a castor seed (*Ricinus communis*); remove the seed coats, cut very thin transverse sections of the endosperm, and defat them by maceration for fifteen to thirty minutes in a mixture of ether and alcohol (equal parts), or in this particular case absolute alcohol; transfer them to alcohol. Mount one in alcohol.

Observe, especially near the thin edge of the section, large ovoid or rounded bodies; they are aleurone grains, but they do not well exhibit their structure when examined in this medium. Irrigate gently with water tinged with iodine; the crystalloid and globoid become visible; the former is coloured yellow; they are embedded in a ground substance, and appear surrounded by a delicate membrane. Irrigate with very dilute potash (0.3 per cent.); the membrane, ground substance, and crystalloid dissolve, the globoid remains undissolved.

Repeat this experiment, using solution of picric acid instead of iodine; the aleurone grains are coloured bright yellow, the globoid appearing of a reddish-orange tint.

Mucilage

Substances of a mucilaginous or gummy nature are very frequently present in the parenchymatous cells of vegetable organs, and are liable to escape detection, as they are usually nearly colourless and exhibit but few distinctive reactions.

These substances, which may be included in the generic term 'mucilage,' occur in a number of varieties differing from one another in their mode of production as well as in their nature and composition. The varying degrees of solubility, the varying extents to which they swell, and the reactions they yield indicate that many mucilages are not homogeneous bodies but mixtures in which sometimes one, sometimes another variety preponderates.

Mucilage may result from a transformation of the cell wall, as is the case with tragacanth, or it may be produced in the protoplasm and deposited on the whole or a portion of the surrounding cell wall in the form of a secondary thickening. The latter is the more common in drugs. Usually the mucilage is deposited in successive layers, and exhibits, therefore, either in the dry state or after suitable treatment, a more or less pronounced stratification. Not unfrequently these layers may be separated from the cell cavity by a delicate wall of cellulose, or even similar walls may separate the layers of mucilage from one another. Hence when such a mucilaginous layer bounded by a cellular wall is swollen by the addition of water, the delicate cellulose wall becomes visible and gives rise to the impression that the mucilage has been contained in a distinct cell. Such, however, is not the case.

All varieties of mucilage agree in being insoluble in alcohol and in glycerin, but, as already observed, they either swell or dissolve in water. In sections examined in glycerin or alcohol the mucilage appears as transparent or granular thickenings of the cell wall, which, however, may be so considerable as to fill the cell cavity. Irrigated with water the mucilage usually swells; with alcohol, it again contracts; with solution of subacetate of lead, it becomes yellowish and granular.

Mucilages have been variously classified. The following may be briefly noticed as introducing the student to the chief distinctive colour reactions.

I. Classification into cellulose, amyloid, and true mucilages.

- (a) Cellulose mucilages; these are coloured blue by chlorzinciodine (mucilage of quince seed).
- (b) Amyloid mucilages; these are coloured blue by iodine alone (mucilage of tamarind seed).
- (c) True mucilages; these are not coloured blue, but pale yellow (mucilage of fœnugrec seed, linseed, marsh-mallow root)

II. Classification into starch mucilages, cellulose mucilages, and gums.¹

- (a) Starch mucilages; these are stained permanently by corallin-soda.

¹ Szyszyłowicz, *Bot. Cent.* xii. 138.

- (b) Cellulose mucilages ; these are stained by corallin-soda, but less permanently.
- (c) Gums are not stained at all.

III. Classification into pectose mucilages and cellulose mucilages.¹

- (a) Pectose mucilages ; these stain with ruthenium red.
- (b) Cellulose mucilages ; these do not stain.

The following reagents may, therefore, be indicated as useful in endeavouring to stain mucilages.

- (1) Chlorzinciodine ; this will colour Tschirch's cellulose mucilages blue.
- (2) Corallin-soda ; this will stain Szyszylowicz's starch mucilages pink.
- (3) Ruthenium red ; this will stain Mangin's pectose mucilages pink.

In each case the section should be examined in the reagent itself.

White Mustard Seed

Source.—The seed of *Brassica alba*, Boiss. (N.O. *Cruciferae*).

Preparation and Examination of Sections.—Before proceeding to cut sections of the seed the student should make himself familiar with its structure. Soak a few seeds in water, during which they will surround themselves with mucilage ; when thoroughly softened, use the dissecting needles to strip the seed coats from one. Examine the kernel. It consists of two folded cotyledons, embracing the small radicle. Cut another seed in half midway between the hilum and the apex ; examine the cut surface with a lens ; it exhibits sections of the cotyledons and radicle surrounded by the seed coats. There is no visible endosperm.

Prepare some seeds for section cutting as follows :

Mix a few with gum and glycerin (see list of reagents), put them on the flat end of a small cork, and let them dry on. The glycerin will prevent the gum from becoming brittle, but

¹ Mangin, *Comptes Rendus*, cxvi. 653.

the mucilage will hold the seeds firmly enough for cutting. Or a single seed may be fixed in pith, with the hilum upwards, so that transverse sections may be obtained.

Cut a number of transverse sections as nearly through the centre of the seeds as possible. Keep them in alcohol.

Seed Coats.—The seed coats may separate from the kernel, but this is of no consequence. Transfer several of the sections of the seed coats to a slide; immerse them in water to dissolve the gum, and remove the watery solution with filter paper; mount in chloral hydrate.

For examination select a section that has passed through the centre of the seed and exhibits sharply defined lines of cells

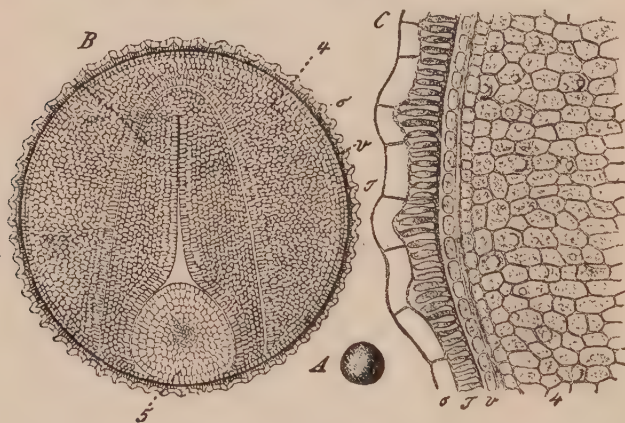


FIG. 99.—Mustard Seed (black). *A*, entire seed, $\times 3$; *B*, transverse section, $\times 65$; 4, the cotyledon; 5, the radicle; *C*, portion of the same, still further enlarged; σ , the mucilaginous epidermis. (Berg.)

without any blurring from outer layers overlapping inner layers, as would be the case with tangential sections. Observe the following layers:

(1) An epidermis (fig. 100, *E*), consisting of large rectangular cells, flattened or sometimes nearly square in transverse section. They average from 40 to 80 μ in length and have very thin walls, the outer one being usually convex. Careful examination shows them to be filled with a transparent, colourless, striated substance, in the centre of which there is a narrow cavity.

Mount a fresh section in alcohol (without treatment with water) and irrigate gently with water, watching the epidermis.

The cells swell and become more distinct. Remove the water and add solution of ruthenium red ; the substance contained in them stains bright pink. It is mucilage, which is deposited on the walls of the cells and swells in contact with water, often exuding in the form of dome-shaped, transparent masses from cells that have been cut by the razor.

(2) A layer usually two cells thick, composed of large parenchymatous cells, the angles of which often exhibit inter-

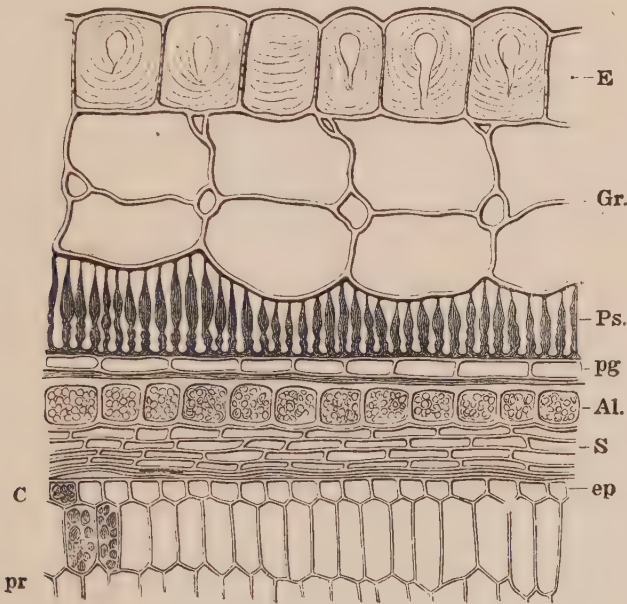


FIG. 100.—White Mustard Seed, transverse section. Al., aleurone layer; C, cotyledon; E, mucilaginous epidermis of seed coats; ep, epidermis of cotyledon; Gr., large subepidermal parenchymatous cells; pg, parenchymatous cells corresponding to the pigment cells of black mustard; pr, parenchyma of cotyledon; Ps., sclerenchymatous palisade cells; S, collapsed cells of nucellus. (Vogl.)

cellular spaces and the walls collenchymatous thickening (fig. 100, Gr.). They are often collapsed, and hence not easily seen, but here and there they are usually distinct and may be made more so by warming the preparation with chlorzinciodine or strong (20 per cent.) solution of potash. They are very easily seen in surface sections.

(3) A single row of palisade cells (fig. 100, Ps.). These vary from 5 to 10 μ in width and from 30 to 40 μ in length. They

are tolerably uniform in length, but nevertheless a slight increase is perceptible at regular intervals, corresponding to the undulating course of the inner walls of the parenchymatous layer above it.

The walls of the palisade cells are pale yellow in colour, the inner and radial being much thickened. This thickening is not uniform, but tapers rather abruptly away in the upper part of the cell, the wall becoming thin, wavy, and often collapsed. The lower thickened part has often an irregular, jagged edge. Although the thickening is considerable, the lignification is but slight in extent.

(4) A thin colourless membrane which is composed of two or three rows of collapsed parenchymatous cells. No cell contents are visible, and indeed little structure can be made out beyond indications of cell lumina (fig. 100, pg). One layer of cells can be made more distinct by caustic potash.

(5) A single row of very conspicuous rectangular cells with rather thick walls (fig. 100, Al.). These cells contain oil and aleurone grains, which can be stained by appropriate reagents; hence the layer is termed the aleurone or proteid layer. In the section under examination the contents will have been altered by the action of the water and chloral hydrate with which the sections have been treated. The cells of the aleurone layer vary from 15 to 30 μ in width, and are about 15 μ in height.

(6) Lastly, there is a colourless hyaline layer composed of several rows of more or less collapsed parenchymatous cells (fig. 100, S). These may be made more distinct by the use of concentrated solution of potash or other reagents that induce a swelling of the cell wall; they generally exhibit rather thin walls, and long narrow cavities without any perceptible contents.

The layers 1, 2, and 3 are probably derived from the integuments of the ovule, while 4, 5, and 6 represent the remains of the nucellus and endosperm.

Kernel.—Fix a seed in pith with the hilum upwards, and cut sections as thin as possible through the centre; they will cut the radicle transversely.

Mount one in chloral hydrate; observe the numerous globules of oil that exude.

Transfer the remainder to ether or a mixture of ether and

alcohol in a small corked tube, and macerate for a few minutes to remove the oil; then pour off the ether, add a little alcohol, and transfer to a dish.

Transfer a few of the defatted sections to a drop of water on a slide, remove the water, and add chloral hydrate.

Examine first the sections of cotyledons. The tissue is thin-walled, and shows a distinct small-celled epidermis on either side (fig. 102 *cot.*). Under the epidermis of the lower surface the cells are elongated to form a varying number of rows of palisade cells; under the upper epidermis the cells are more rounded. In the centre the procambial strands formed of very small cells can be seen.

The radicle exhibits an epidermis, next to which there is a cortical tissue of cells that have rather thicker walls than the cells of the cotyledons and exhibit intercellular spaces. In the centre is the rudimentary stele.

Sketch a portion of cotyledon and radicle.

Next examine the cell contents. Mount one or two very thin defatted sections in a saturated solution of picric acid; after five minutes examine the cells at the thin edges of the sections. Observe that they are filled with irregularly oval or rounded bodies that are stained yellow and appear granular. They are aleurone grains. They are often very conspicuous if the stained section is mounted in glycerin (fig. 102, *al.*). Sketch a few. Irrigate very gently with dilute solution of potash (0.3 per cent.); they rapidly dissolve, each leaving a number of minute granules, visible with difficulty, behind. These granules are globoids.

Mount another section direct in glycerin (without water); the aleurone grains are quite distinct; they will dissolve as before in dilute potash.

The aleurone grains can also be stained with other reagents, among which iodine is perhaps the best, as it distinguishes them from other substances, more particularly from starch grains. Iodine water, or water tinged yellow with iodo-potassium iodide, may be used, and the grains subsequently dissolved by very dilute solution of potash.

Mount a section of the kernel in water and irrigate with solution of potash; the section acquires a yellow colour. This reaction is characteristic of mustard seed.

Mount another section in Millon's reagent; the cell contents

gradually acquire a brick-red colour (reaction for proteid matter). Some of the cells which are larger than their neighbours are distinguished by the brighter colour they assume with Millon's reagent ; these are said to contain the myrosin.

Disintegration of the Seed Coats.—Soak some seeds in water for a few hours, and also some others in solution of potash for

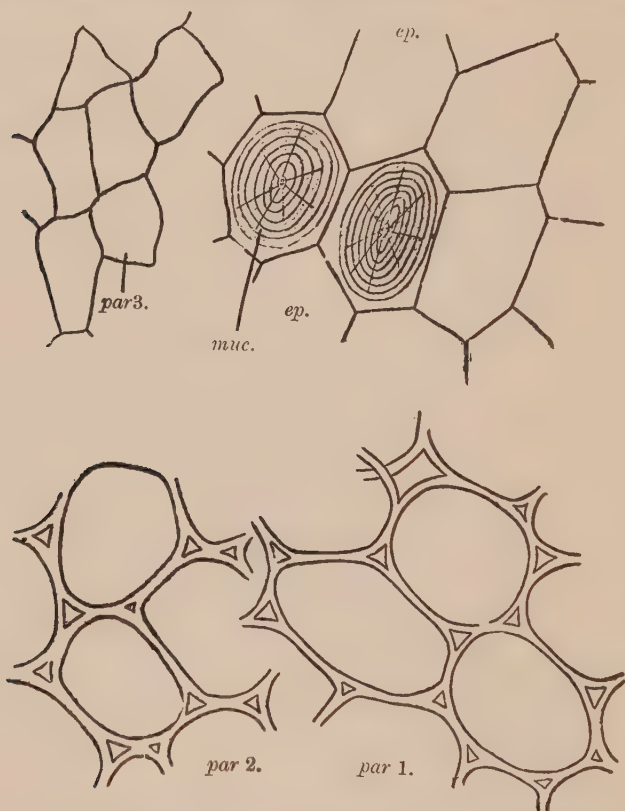


FIG. 101.—White Mustard Seed, from the powder. *ep.*, epidermis (in two cells the mucilage, *muc.*, is figured); *par 1.*, the upper of the two layers of parenchyma; *par 2.*, the lower layer; *par 3.*, cells of the layer pg in fig. 100.

an hour or more. Cut one of each open, and remove the kernel. Hold a piece of the seed coat firmly with a needle, and scrape the surface vigorously with the sharp edge of a glover's needle ; disintegrate it as much as possible by both cutting and scraping. Examine the *débris* thus obtained in solution of potash. Search for and identify the layers 1, 2, 3, 4, 5, and 6

that have been found in the transverse sections. They will now present the same appearance as they will in the powdered drug, and hence this examination is most important.

(1) Epidermis (fig. 101, *ep.*): the cells are easily identified by their large size (45 to 60 μ in diameter), polygonal, transparent, mucilaginous contents, and thin walls. The mucilage

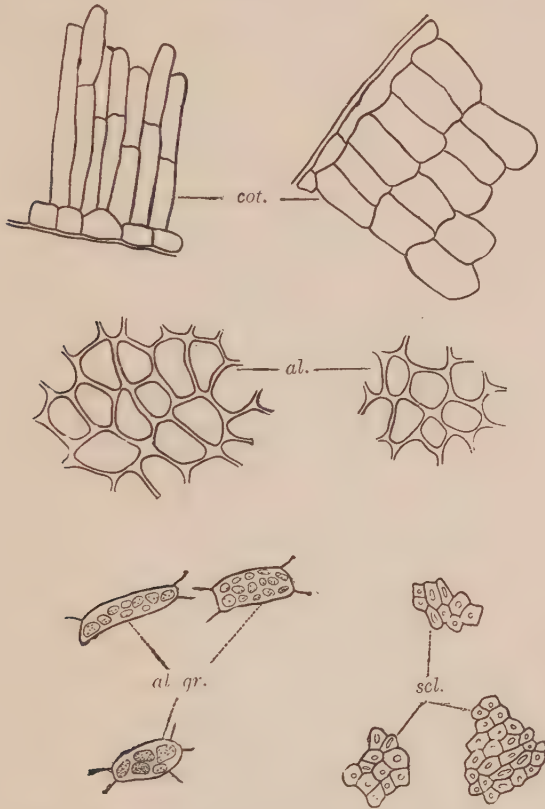


FIG. 102.—White Mustard Seed, fragments from the powder. *cot.*, cotyledons; *al.*, cells of the aleurone layer; *al. gr.*, cells of cotyledon with aleurone grains; *scl.*, palisade cells.

exhibits circular striations and a small central cavity from which delicate lines run to the wall. It can be stained, if necessary, with solution of ruthenium red.

(2) This layer usually adheres to others, especially to No. 1: it consists of large rounded cells with rather thick walls and intercellular spaces; the walls are often thickened near the

angles. By focussing down the presence of a second similar layer can often be determined (fig. 101, *par 1*, *par 2*).

(3) The palisade cells present their surface view, which is quite different from the profile. The fragments of this layer are easily distinguished by their pale yellow colour. On examination they appear as small polygonal cells with thick walls and small cavities. The thick walls are the thickened radial walls seen in the section (fig. 102, *sc1*). If the cells are lying with their outer surface upwards—that is, in the position in which they exist in the seed—then on focussing carefully upwards the outlines of the thin upper part of the walls may often be detected as a delicate polygonal network. Sometimes layer 2 adheres to the palisade layer, and its cells can then be distinguished on still further raising the focus; in fact, the presence of this layer is often useful as identifying the upper surface of the palisade layer.

(4) Layer No. 4 generally adheres closely to No. 5 (the aleurone layer), and in this peculiarity, as well as in the shape of its cells, it resembles No. 6. The cells are polygonal, very thin-walled, and very inconspicuous (fig. 101, *par 3*).

(5) The aleurone layer is usually easily found; the cells are rather thick-walled, polygonal, or rounded, and have granular contents (fig. 101, *al*).

(6) This layer consists of several rows of very thin-walled collapsed cells, and generally adheres firmly to the aleurone layer.

Of these layers the student should particularly study Nos. 1, 2, and 3, as these are very characteristic of white mustard. Most cruciferous seeds resemble white mustard in their general structure, especially in the presence of a palisade layer, and the details of these tissues should therefore be carefully noted (compare black mustard).

Examination of the Powdered Seed.—Prepare some powder from white mustard seeds by crushing them in a mortar; free this coarse powder from fixed oil by washing it with ether or any similar solvent; dry it first by exposure to the air, and finally, for an hour or so, in an air oven, then powder again, and pass the powder through a No. 60 sieve.

Examine the powder first for aleurone grains. Mount in alcohol. The preparation will contain abundance of the minute isolated aleurone grains.

Mount a little of the powder in solution of picric acid ; the aleurone grains stain yellow ; many are scattered throughout the preparation, others are still enclosed in the cells. They are usually very distinct if a small drop only of picric acid is used and, after five minutes, a drop of glycerin is added.

Irrigate a stained preparation with very dilute potash ; the grains dissolve at once, leaving behind a number of minute globoids.

Mount a preparation in water and irrigate with chloral iodine ; observe the presence here and there of a minute starch grain or a group of a few ; these are probably from seeds that are not quite ripe.

Moisten a little of the powder with alcohol and irrigate with potash ; observe the yellow colour which is produced by fragments of the kernel. Examine this preparation carefully for tissues. The mucilaginous epidermal cells are easily found under a low power, as they stand out against the yellowish liquid, the mucilage remaining colourless and strongly refractive.

The palisade tissue is also very easily identified by its colour and its cells. Layer No. 2 often adheres to this or to the epidermis.

The aleurone layer is colourless, and bears attached to it usually both layers of collapsed parenchyma (No. 4 and No. 6), one on either side.

Much of the *débris* of the cotyledons and radicle can be found in the shape of small masses of delicate parenchymatous cells.

Mount a little of the powder in chloral hydrate ; examine in this preparation more particularly the fragments of the cotyledons and radicle ; the cell walls of the latter are rather thicker than those of the cotyledons and there are intercellular spaces.

Mount a little also in ruthenium red ; the mucilage stains pink.

Examination of Commercial Mustard.—In preparing the table mustard of commerce the seeds are crushed and the seed coats are almost entirely removed by sifting. Mustard consists, therefore, chiefly of the powdered cotyledons and radicle, with occasional fragments of the seed coats.

In examining it the following method will be found advantageous :

Defat about 2 grammes of the powder, drain well, and dry by exposure to the air. Reserve a portion of this for examination, as directed for the powdered seed.

Take about 0·5 gramme of the defatted powder and suspend it in a weak (0·5 per cent.) solution of caustic potash, which will dissolve the ground substance of the aleurone grains and clear the tissues. Separation of the cellular *débris* by subsidence is tedious, as the liquid is rather viscid, and recourse must be had to the centrifuge. Wash the deposited cell *débris* once with water, and separate again. The fragments of the seed coats are usually deposited first, as they are comparatively heavy and can often be easily distinguished in the point of the tube.

Examine this deposit in dilute glycerin or chloral hydrate. The various tissues are exceedingly clear, and can easily be identified. Any cells or tissues derived from foreign seeds can easily be detected, and are much more easily found in the powder prepared as indicated than in that which has not been so treated.

In exceptional cases it may be desirable to endeavour to remove the delicate parenchymatous tissue and thus concentrate any more resistant cells or tissues, such as sclerenchymatous cells, bast fibres, &c., into a smaller compass. This may be effected by warming the defatted powder with nitric acid and potassium chlorate, stopping the operation as soon as the parenchymatous tissue is judged to be destroyed, and then separating by the centrifuge the tissues that have resisted oxidation. Among these will be the sclerenchymatous layer of the seed coats in a more or less altered condition. The presence of any sclerenchymatous cells, &c., would indicate impurity or adulteration.

Black Mustard Seed

Examine this seed in the same way as white mustard, and note the following differences :

- (a) The mucilage in the epidermal cells swells less and is less conspicuous ; the striations are not marked.

- (b) Layer No. 2 (fig. 103, *m*) consists of a single row of cells which do not exhibit the intercellular spaces and collenchymatous thickenings characteristic of those of white mustard.
- (c) The palisade cells are dark yellowish or reddish-brown in colour, and are conspicuously long and short at intervals; this irregularity makes itself perceptible in indistinct reticulate outlines on the surface view of the palisade tissue.

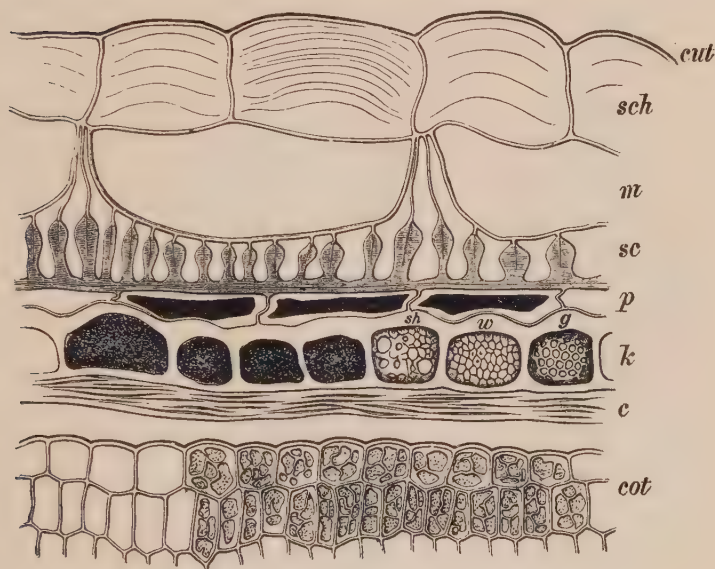


FIG. 103.—Black Mustard Seed, transverse section. *c*, collapsed remains of nucellus; *cot*, cotyledon; *cut*, cuticle; *k*, aleurone layer; *m*, large subepidermal cells; *p*, pigment cells; *sc*, sclerenchymatous (palisade) cells; *sch*, mucilaginous epidermis. (Tschirch.)

- (d) The cells of layer No. 4 (fig. 103, *p*) contain a dark reddish-brown amorphous substance.

The cells of the cotyledons and embryo and their contents do not differ in appearance from those of white mustard, and the microscope would be unable to distinguish between them if the husk were entirely removed. This, however, is never completely effected, and small fragments of the seed coats are always to be found in the table mustard of commerce.

Linseed

Source.—The seed of *Linum usitatissimum*, Linn.

Preparation.—The method to be followed in the examination of linseed is similar to that for mustard, but in this case the seed should be fixed in cork for section cutting. The seed coats are very liable to separate from the cotyledons, and often from the endosperm also, but this need not cause any inconvenience.

Examination of Transverse Section.—The transverse section shows the following tissues :

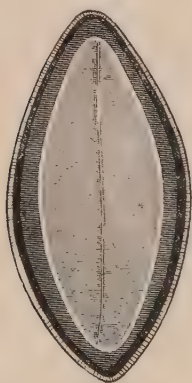


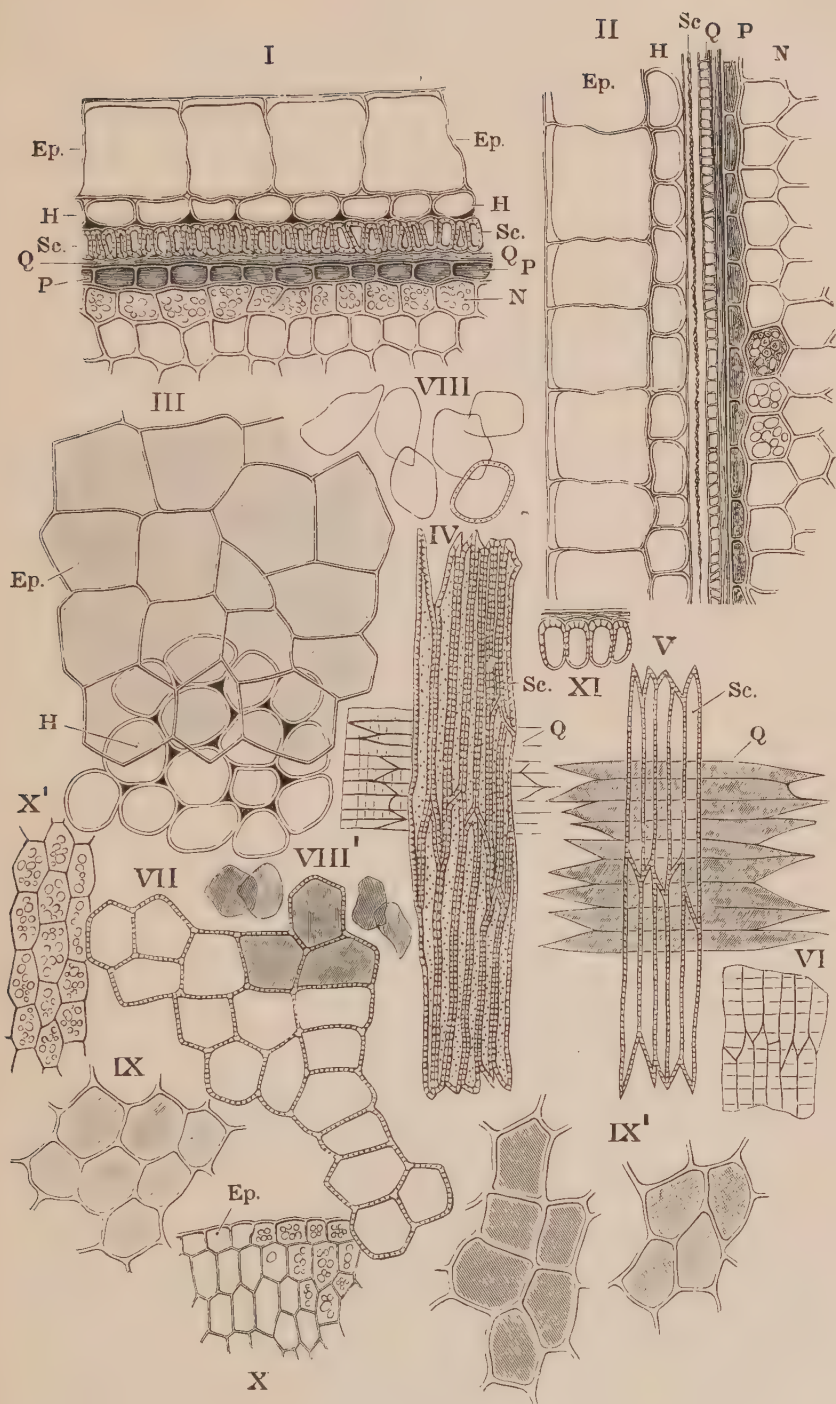
FIG. 104.—Linseed, transverse section, showing the seed coats, endosperm, and cotyledons. Magnified. (Moeller.)

- (1) *Epidermis*, consisting of large cells (width about $30-45\ \mu$), which contain mucilage deposited on the outer and radial walls. In contact with water the mucilage swells considerably, and often exhibits very distinct stratification, which, however, is quite different from that of white mustard ; it stains red with ruthenium red.

The epidermal cells have very thin radial walls, which suddenly thicken a little near the inner tangential wall ; the outer tangential wall is rather thicker, and is provided with a distinct cuticle. (Fig. 105, Ep., in which the mucilage is not shown.)

- (2) *Parenchymatous Layer*, consisting usually of two rows of cells (diameter, $20-25\ \mu$) which, however, are often partly collapsed ; they have rather thick walls, and exhibit intercellular spaces. Towards the sharp edge of the seed there are about five rows of such

FIG. 105.—Linseed. I, transverse ; and II, longitudinal sections of seed coats and subjacent endosperm cells ; Ep., epidermis (mucilage not shown) ; H, sub-epidermal parenchyma (in this case one layer only) ; Sc, sclerenchymatous cells ; Q, thin-walled cells crossing these at right angles (compare IV and V) ; P, pigment cells ; N, cells of endosperm ; III, surface view of mucilaginous epidermis, Ep, and subjacent parenchyma H ; IV and V, surface view of sclerenchymatous cells with thin-walled parenchymatous cells crossing them ; VI, the latter cells alone ; VII, surface view of pigment layer ; VIII, isolated cells of same ; VIII', brown masses of contents of these cells ; IX, IX', endosperm cells in surface view ; X, section through the margin of the cotyledon ; XI, group of sclerenchymatous cells (figs. III and IV from the powder). (Vogl.)



cells ; here they have thinner walls, and are more tangentially elongated. Near one edge the raphe can generally be detected as a small fibro-vascular bundle in this tissue. (Fig. 105, H, in this case one layer only of cells.)

- (3) *Sclerenchymatous Layer*, composed of radially elongated cells of yellowish-brown colour. These cells vary in size and appearance in different varieties of linseed, and are usually larger and more strongly radially elongated near the edge of the seed than they are on the flat side. Their walls are thickened, pitted, and lignified. (Fig. 105, sc.)
- (4) *Hyaline Layer*.—This is narrow and colourless, and exhibits indications only of cell lumina. (Fig. 105, q.)
- (5) *Pigment Layer*, which is very conspicuous. It consists of a single row of flattened parenchymatous cells, each of which is completely filled with a homogeneous, dark reddish-brown, amorphous mass. (Fig. 105, p.)
- (6) *Kernel*, consisting of a narrow endosperm, two cotyledons, and a small radicle.

Both cotyledons and endosperm consist of delicate parenchymatous cells containing aleurone grains and fixed oil.

The cells of the endosperm are approximately isodiametric, and have comparatively thick walls (fig. 105, ix, ix'.) The aleurone grains are small and irregular, and do not well exhibit either globoid or crystalloid. In addition to aleurone grains, the cells contain oil, which readily exudes on treatment with chloral hydrate.

The cells of the cotyledons are elongated and have very thin walls (fig. 105, x.) They contain very characteristic aleurone grains.

Examination of the Aleurone Grains.—Mount a very thin section in glycerin ; the aleurone grains show very distinctly ; they are ovoid in form, averaging 10 to 15 μ in length, and contain a large globoid at one end.

Stain a section with iodine or picric acid ; the globoid and

two or sometimes only one large, obscurely angular, crystalloid can be seen.

Irrigate with dilute solution of potash; the ground substance and crystalloids dissolve at once, leaving only the large rounded globoids. These are conspicuous and characteristic, and serve as a means of identifying fragments of the cotyledon of linseed even after treatment with ether followed by dilute potash.

Isolation of the Tissues.—The tissues of which the seed coats consist must be isolated and examined as those of mustard seed were. Split one or two seeds open, and remove the kernel. Macerate the seed coats for an hour or more in solution of potash; then vigorously scrape and tease out the tissues.

- (1) The epidermal cells are easily identified; they are large, polygonal or oblong, and thin-walled, and contain or are surrounded by transparent mucilage (length, 30 to 75 μ , mostly 40 to 60 μ). (Fig. 105, III, Ep.)
- (2) The parenchymatous layer (2) usually adheres firmly to the sclerenchymatous layer (3), and is often covered by the epidermis. The cells are rounded and rather thick-walled, with intercellular spaces. Diameter about 30 μ . One row of cells is always distinctly visible, but the second is not easy to detect. Those from the edge of the seed are larger and thinner; such cells cover larger and paler sclerenchymatous cells. (Fig. 105, III, H.)
- (3) The sclerenchymatous layer is very conspicuous, and is almost always associated with 2 and with 4. It has a brownish-yellow colour, and consists of very long (200 μ and upwards), very narrow (often 4 to 5 μ) fibrous cells with thickened, lignified, pitted walls. The cavities of the cells are very narrow, and hence their shape is not very readily seen. Separation by maceration with Schultze's mixture assists in the correct interpretation of them. (Fig. 105, IV, sc.)
- (4) This, the hyaline layer, always remains firmly attached to the sclerenchymatous cells, and is best seen at the torn edge of the latter, from which it often projects a little. It consists of long, narrow, colourless parenchymatous cells with very thin walls, and these cells

are themselves crossed at right angles by another similar layer, which, however, can be seen only here and there. (Fig. 105, iv, q.)

Layers 2, 3, and 4 almost always occur together, and are very characteristic of linseed.

- (5) The pigment layer is very easily found. The cells are polygonal or oblong (20 to 30 μ), and have straight or slightly curved, rather thick, pitted walls. They are completely filled with a deep brown, homogeneous, amorphous mass, which, however, often falls out intact from torn cells; such cell contents can be found in every preparation. (Fig. 105, vii, viii, viii'.)

Examination of the Powder.—Defat some powdered linseed as directed for mustard.

Moisten a little on a slide with alcohol; allow it to nearly dry, and then add a small drop of solution of picric acid; after five minutes add a drop of glycerin, mix well, and examine. The aleurone grains stain yellow, and can be readily identified (the yellow colour is very slowly removed by the glycerin). Those of the cotyledons are more conspicuous than those of the endosperm. Irrigate with dilute solution of potash, and observe the globoids that are left.

Mount a little in chloral hydrate (after alcohol). The tissues of the seed coats are usually very distinct. The sclerenchymatous layer is the most conspicuous. With it are commonly associated the parenchymatous layer and epidermis above, and the delicate parenchyma below; the latter tissue is best seen at the edges of the fragment, where it projects beyond the sclerenchyma; the other layers can usually be seen by suitable focussing.

Very conspicuous also are the quadratic cells of the pigment layer, with their pitted walls; the brown amorphous masses of contents are also easily found.

The parenchymatous tissue of the cotyledons can be identified particularly easily by the numerous large rounded globoids left after solution of the ground substance and crystalloids; these are very conspicuous. Similar globoids are to be found scattered over the field.

The cells of the endosperm are less elongated; they have rather thicker walls, and less conspicuous globoids.

Mount a little in ruthenium red; the masses of mucilage stain pink.

Nux Vomica Seeds

Source.—The seeds of *Strychnos Nux-vomica*, Linn.

Preparation and Examination of Sections.—Split some seeds in half and soak them, together with a few whole seeds, in water for twelve or twenty-four hours. Expose also some split seeds to a moist atmosphere for twenty-four hours. Take a suitably softened half-seed, and cut it into two semicircular pieces by an incision passing through the centre. As the hairs assume a radial arrangement, this incision will be parallel to them, and this can easily be determined by examination with a lens. Now cut a narrow strip by two incisions at right angles to the radial cut; sections from the flat end of this strip will then be parallel to the direction which the hairs assume. Embed this strip in pith, and cut sections, taking care that the razor follows the hairs from base to apex, and transfer them to alcohol.

Mount a section in water. The outermost layer consists of the epidermal cells, which have developed into remarkable hairs; these are bent over near the base, and closely appressed to the seed, thus giving it its silky appearance. These hairs cannot, however, be satisfactorily studied from a section, as many of them will have been cut, and in any case the basal part is indistinct. They will be more closely examined later.

Following the epidermis is a narrow brown layer in which indications of collapsed cells can be discerned. This layer can be isolated by warming with potash, and is then seen to consist of ill-defined, delicate, thin-walled, polygonal cells.

These two layers comprise the whole of the integuments of the seed, and are directly followed by the endosperm, which is next to be examined.

In a section mounted in water observe that the endosperm cells are large and have very thick, strongly refractive walls. Those at the periphery are smaller than the inner ones, and more elongated, forming a kind of palisade. The cells have granular contents.

Cut some sections from a seed that has not been soaked in water; flatten them out as well as may be, and mount in alcohol. In the cells irregular angular granular masses can be seen, often two, three, or four together in one cell. These

are the aleurone grains. Irrigate with iodine water; the cell walls swell; the aleurone grains acquire a yellow colour and

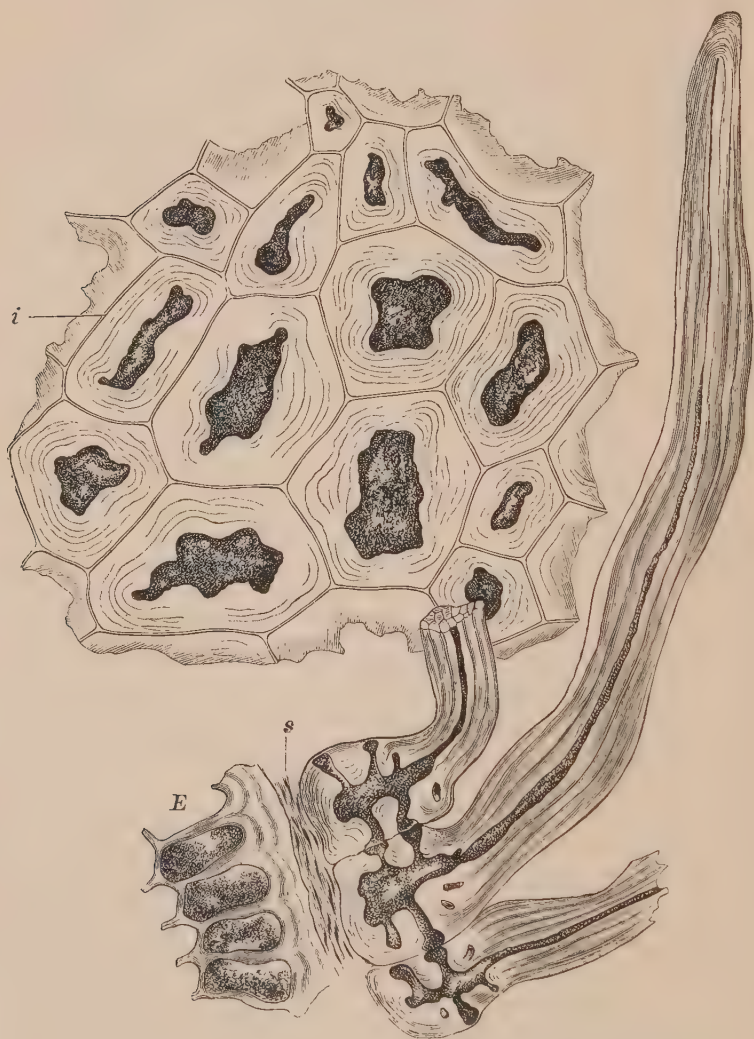


FIG. 106.—*Nux Vomica*. The upper portion is a section of the endosperm, the cell walls of which have been swollen by water; *i*, primary cell wall. The lower portion is a section at the margin; *E*, the outer row of endosperm cells; *s*, the seed coat to which the hairs are attached. (Moeller.)

become more distinct. Irrigate with dilute potash; they dissolve, leaving behind a few minute globoids.

Mount another section in water and irrigate with solution of potash; the cell contents assume a yellow colour, due probably to the caffeotannic acid present.

Mount a section in alcohol. Focus under the low power. Irrigate it with water; the cell walls swell distinctly. Warm the section till the water just begins to boil; the cell walls swell still more strongly, often completely obliterating the cavity and squeezing the contents out.

Stain another section with solution of iodopotassium iodide, wash, and irrigate with concentrated sulphuric acid; the cell walls assume a blue colour. These tests indicate that the cell walls are composed of a mucilaginous variety of cellulose.

Next study more carefully the hairs.



FIG. 107.—*Nux Vomica*. I, hairs viewed from below, showing the transverse sections of bases; II, base of hair viewed from the side. $\times 240$.

Shave off from the surface of a seed the hairy epidermis (with part of the subjacent endosperm). Digest for a few minutes with potassium chlorate and nitric acid, wash, and transfer to spirit.

Examine a fragment in glycerin, placing it with the hairy side downwards. In favourable pieces the outlines of the epidermal cells can be seen; they are usually very irregular, wavy, or jagged, and often bear knob-like projections, which can be seen by focussing upwards.

Tease out another part into its component hairs. Observe their remarkable shape. The basal part is very irregular (compare with previous preparation), often bearing projections below and oblique pits on the sides. The upper part is drawn out into a very long hair ($1,500 \mu$), the wall of which bears on

its inner surface a number of nearly parallel thickened bands which anastomose but little, and finally meet in the rounded apex of the hairs; these bands are lignified. In many hairs the oxidising mixture has destroyed the intervening strands of cellulose, and the hairs fray out into their component bands towards the apex; some, however, will be intact.

Mount a section (from a seed that has not been immersed in alcohol) in water; remove the water, and irrigate with sulphovanadic acid; the contents of the cells rapidly assume a violet colouration (reaction for strychnine).

Examination of the Powder.—The best medium for a preliminary examination is alcohol. In it the powder shows fragments of endosperm cells, with their very thick walls. Water makes these swell, and their structure becomes clearer. Irrigation with potash now produces a bright yellow colouration, dissolves the aleurone grains, which, however, are not any time very conspicuous, and further swells the cell walls. Fragments of the hairs, especially portions of the isolated bands, are numerous, but they are not very conspicuous. The characteristic basal part of the hair is not easy to find.

Chloral hydrate clears well, and makes the thick endosperm walls almost invisible. Fragments of the hairs are much more easily seen, and an occasional basal part may be found. Staining with phloroglucin and hydrochloric acid also directs attention to the hairs. Picric acid will perform a similar office for the aleurone grains.

A good method of examination, applicable under certain conditions, is to treat a little of the powder with potassium chlorate and nitric acid, wash, and examine the deposit. The endosperm is destroyed, and now fragments of hairs, the basal portion in various positions, &c., may be easily found and identified.

Areca Nut

Source.—The seeds of *Areca Catechu*, Linn.

Preparation and Examination of Sections.—Select one or two areca nuts that have part of the yellowish inner layers of the pericarp adhering to them and soak them for forty-eight hours in water or till they are sufficiently soft to cut. Cut transverse sections from the outer part of a seed, taking care

to include the remains of the pericarp. Transfer the sections to alcohol.

Mount one in chloral hydrate.

The outermost layer consists of elongated oval cells, with slightly thickened, pitted, and lignified walls. These cells vary in size, but often measure about 120 to 150 μ in length and 18 to 20 μ in breadth. They exhibit intercellular spaces, and easily separate from one another; hence they may be found loose, or nearly so, and hence also the surface of the remains of the pericarp is scurfy.

This layer is usually from two to six cells wide, and is bounded on the inner side by a single row of parenchymatous

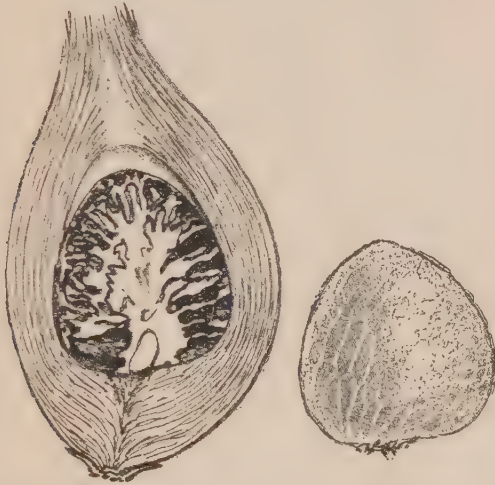


FIG. 108.—Areca Nut. A, vertical section of the fruit and seed, showing the fibrous pericarp of the former and ruminant albumen of the latter; B, seed. Natural size. (Bentley and Trimen.)

cells which present a nearly square or slightly radially elongated section. These cells vary from 10 to 15 μ in width, and have pitted, strongly lignified walls. Surface sections show them to be polygonal and approximately isodiametric. This layer is the inner epidermis of the pericarp, and hence the tissues hitherto observed do not constitute part of the seed proper.

Following close upon the inner epidermis of the pericarp are the seed coats. These average about 300 μ in thickness, and are rather sharply differentiated into two layers, an outer thick-walled and an inner thin-walled one. The cells of the

outer layer present an oval, rounded, or elongated section due to the irregular direction of the cells ; between them there are often intercellular spaces, or even cavities of considerable size, caused by the destruction of delicate parenchymatous tissue which originally filled them. Those cells that abut on the pericarp are often compressed or collapsed, and hence not well defined ; here and there elongated narrow cells can be found ; the epidermis is seldom distinguishable. The cells next to

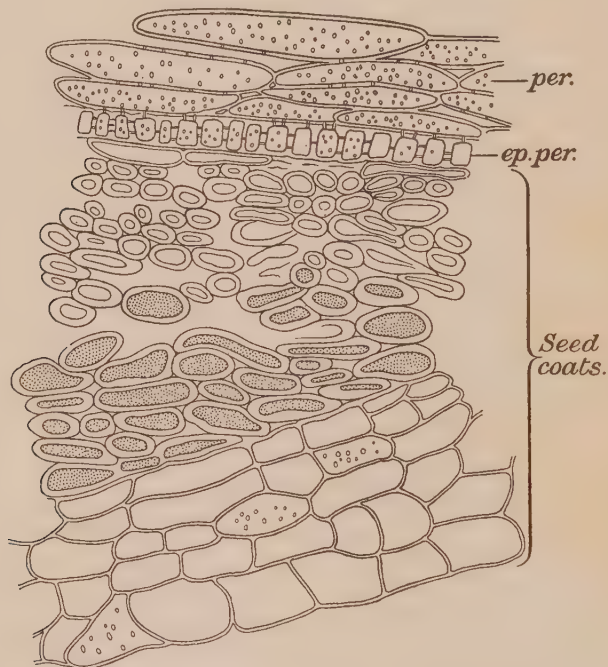


FIG. 109.—Areca Nut, transverse section of seed coats. $\times 240$. *per.*, inner layers of cells of pericarp ; *ep. per.*, inner epidermis of pericarp.

these present rounded, oval, or even elongated sections ; they measure from 10 to 15 μ in diameter, and have comparatively very thick walls.

Towards the centre of the seed coat the cells are larger, measuring 20 to 60 μ in length ; the lumina are larger and the walls are not so thick in proportion to the size of the cells as those of the smaller cells. All the cells of this outer thick-walled layer, especially those of the inner part of it, have dark reddish-

brown cell contents, and often reddish-brown walls; this imparts to the seed coat its characteristic colour.

In order to obtain an insight into the actual shape of these cells it is necessary to separate them. This can very easily be done by gently scraping off the seed coat, moistening the powder so produced with alcohol, and finally examining in glycerin. The cells exhibit the greatest variety of outline; some are very long and narrow, many are elongated and have moderately thick pitted walls, others are nearly isodiametric &c. They are not regularly arranged, but often interlace, thus producing the varying sections shown in the illustration. The walls are not striated, and often irregularly thickened.

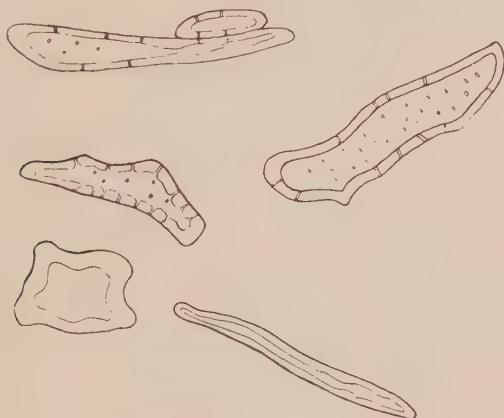


FIG. 110.—Areca Nuts. Cells isolated from outer part of seed coat. $\times 200$.

The inner thin-walled part of the seed coat consists of larger cells with thinner, pitted walls, and contents that are less deeply coloured. Tissue of this nature, but often consisting of smaller and more or less collapsed cells, constitutes the main portion of the ruminations which penetrate the endosperm. The cells, especially those abutting on the endosperm, contain a homogeneous reddish-brown substance that reacts for tannin. At the point where each of these processes diverges from the seed coat there is usually a rather large fibrovascular bundle.

The cells of the endosperm are polygonal and nearly isodiametric, varying very much in size (30 to 120 μ , many about 60 μ). They have very thick cellulose walls (10 μ), which swell in contact with water; the pits are large and rounded (6 to 12 μ),

and often resemble bordered pits in section. They contain a granular proteid matter, which stains yellow with iodine and dissolves in very dilute potash. Many crystals of fat can be distinguished; these melt when warmed, and then stain with appropriate reagents.

Examination of the Powder.—The best medium in which to clear the powder is chloral hydrate; it gradually dissolves the dark red-brown cell contents.

The principal constituents are:

- (a) Fragments of the endosperm; these are colourless, and easily recognised by the very remarkable thickness of the wall and by the large pits. Even small fragments of the cell wall are easily identified. This tissue constitutes the major portion of the powder.
- (b) Fragments of the processes from seed coats. These are generally recognisable by their pale or dark brown cell contents (in so far as they have not been removed). The cells vary a little in shape, but their thin pitted walls and red-brown contents distinguish them.
- (c) Inner portion of seed coat: the cells of which these are composed resemble those of the ruminations, but they are usually comparatively free from cell contents, are larger, and have rather thicker walls.
- (d) Outer portion of seed coat: these cells are very characteristic; they exhibit the most varied shapes.

Cocoa.

Source.—The seeds of *Theobroma Cacao*, Linn.

Examination of the Kernel.

Endosperm.—Remove the shells from a few cocoa beans, and soak the kernels in water; reserve the shells.

Examine the surface of the cotyledons; note the soft pale membrane that adheres to it and also penetrates the folds of the cotyledons. This is the remains of the endosperm.¹ Remove a portion of that which is on the outside, mount in water, and examine. There is only one layer of cells, those of

¹ Tschirch, *Anatomischer Atlas*, p. 22.

the epidermis, to be distinctly seen ; these are polygonal, about 20 to 30 μ in diameter, and contain a greyish granular substance, occasional small crystals of calcium oxalate, and sphaerocrystalline masses of fat ; adhering to the inner surface of this membrane are remarkable scattered hairs ; they are club-shaped, and consist of a single or in the upper part double row of short rounded cells containing brown granules. These hairs may be 100 μ long and 20–30 μ wide ; they are developed from the epidermal cells of the cotyledon, but break off and adhere to the remains of the endosperm. The rest of the membrane consists of a confused mass of delicate collapsed parenchyma.

The fatty nature of the sphaerites above mentioned may be ascertained by their melting into globules when warmed, and by their acquiring a brown colour when irrigated with osmic acid.

That part of the endosperm which penetrates between the folds of the cotyledons is similar to that which covers them externally, but is devoid of the epidermis.

Cotyledons. — Remove the endosperm from part of the cotyledons, and from the surface of the latter cut very thin

surface sections ; mount them in glycerin, keeping the epidermis uppermost. Examine the epidermis. It consists of polygonal, isodiametric, or oblong cells measuring about 15 to 30 μ , with thin walls. Conspicuous among the contents are little rounded granules of deep reddish-brown colour ; to these the dark colour of the outer layer is due.

Examine a transverse section of the outer part of the cotyledon ; the epidermal cells are flattened ; they are characterised by the reddish-brown granules they contain. Below the epidermis the cells are polygonal, about 30 μ in diameter, and appear rather thick-walled ; this, however, is partly due to an adhering

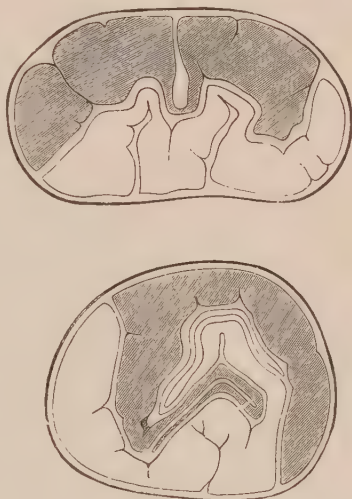


FIG. 111.—Cocoa Seeds. Sections showing the folding of the cotyledons. (Tschirch.)

layer of protoplasm, which can be stained yellow by iodine. Chloral hydrate clears the section well, so also does chloral iodine ; in the latter reagent the cell walls appear thin, and in each cell numerous small starch grains stain blue. Mount a section in water, tearing it so as to liberate the starch grains, and examine them ; they are very small and mostly rounded ; many are simple, but some are compound.

Examine more closely a section mounted in water. In addition to starch grains most of the cells contain large transparent colourless masses which show a more or less distinctly radiate structure. Irrigate a section with solution of osmic acid ; they slowly acquire a brown or finally nearly black colour. Warm a section in water ; they melt to globules. These (and other) tests indicate them to be crystalline masses of fat.

Boil a section gently in chloral hydrate for a few moments ; cool, and if necessary add more chloral hydrate to replace that which has been lost. The starch, fat, and remains of protoplasm dissolve more or less completely. Here and there small crystals of calcium oxalate can be detected.

Defat some sections by maceration in ether-alcohol, wash with alcohol. Mount one in water, and irrigate with iodine water. The distribution of the starch can now be well seen. Sometimes the aleurone grains also will be visible after staining with iodine, but they are not easily seen. They are usually small, and contain a comparatively large globoid in each.

Examine again the section mounted in water ; observe here and there cells or groups of cells containing a reddish amorphous substance. This is cocoa-red. It dissolves in sulphuric acid and in chloral hydrate with red colouration.

The presence of the following cell contents has therefore been determined in the tissue of cotyledon.

- (a) Fat in crystalline masses in most of the cells.
- (b) Starch grains, very small, simple or compound, in most of the cells.
- (c) Calcium oxalate crystals, here and there.
- (d) Aleurone grains, small with large globoid, not easily detected.
- (e) Cocoa red, restricted to isolated cells or groups of a few cells.

Examination of the Shells

Sections.—Expose some cocoa shells to a moderately moist atmosphere until they lose their rigidity, but not longer, as they are apt to become inconveniently moist. Cut transverse sections between pith, transfer to alcohol, mount in chloral hydrate.

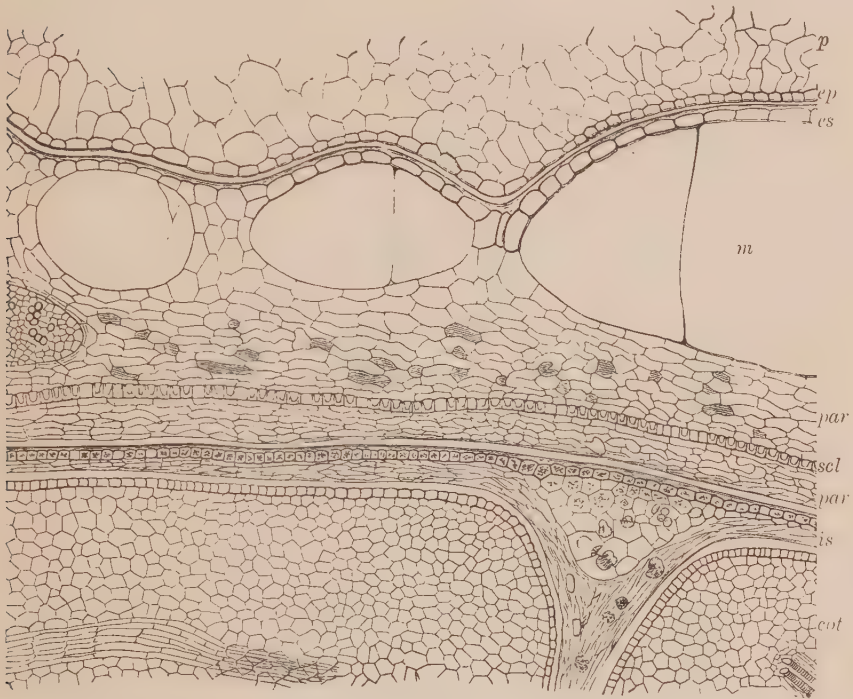


FIG. 112.—Cocoa Seed, transverse section through the periphery of the seed, the seed coats, and the adherent pulp. *cot*, cotyledon; *ep*, inner epidermis of pulp of pericarp; *es*, outer epidermis of seed coat; *is*, remains of endosperm which penetrates between the cotyledons; *m*, mucilage; *par*, parenchymatous tissue of seed coats; *p*, pulp of pericarp. $\times 45$. (Tschirch.)

The sections are very narrow. On the outside there is a confused tissue, in which tubular hyphæ of fungi, numerous minute rounded isolated cells (yeast cells), and occasional crystals can be detected. This is followed by a layer of collapsed cells several rows thick, which show but little structure in sections, but in favourable surface preparations can be seen to be long.

tubular, and very thin-walled ; on the inner side this tissue is bounded by a row of cells, which are more or less conspicuous by reason of the brown colour of their walls. These cells are very small (about 4 to 6 μ in tangential diameter), rectangular, and somewhat flattened in section ; they constitute the inner epidermis of the pericarp of the cocoa fruit (fig. 112, *ep*). The latter contains when ripe a scanty pulp, part of which adheres to the seed throughout the processes of fermentation and drying, during which the fungi and fermentative cells which were observed on the exterior are developed. All the tissue up to and including the epidermis of the pericarp, although always found on the outer surface of the shell, forms no part of the seed coats. The latter consist of the following layers :

- (a) The epidermis of the outer seed coat (fig. 112, *es*) ; the cells are pale in colour, and much collapsed ; hence they are often only indistinctly visible in section, but they can be made more distinct by bleaching the section with solution of chlorinated soda. They are much larger than the epidermis of the pericarp, measuring about 30 μ in tangential diameter.
- (b) Single row of very large mucilage cells, very conspicuous by reason of the large quantity of transparent yellowish mucilage secreted by them (fig. 112, *m*). The cell walls are very thin, and most of them have broken down so as to form large oval cavities separated from one another by transverse belts of three or four rows of parenchymatous cells. Ruthenium red colours the mucilage brilliant pink.
- (c) A mass 150–200 μ thick of collapsed parenchyma, through which large fibro-vascular bundles run ; these bundles are rich in spiral vessels varying in size from 5 to 15 μ .
- (d) A single row of small cells about 10–15 μ in tangential diameter, exhibiting a flattened section and a horse-shoe thickening on the inner and radial walls. This layer is the inner epidermis of the outer seed coat (fig. 112, *sc*l).
- (e) The tissue following this is the inner seed coat. It consists of collapsed parenchyma in which there is no perceptible differentiation.

Surface Preparation.—The next step is the study of these cells and tissues in surface preparations.

From some of the same material cut successive surface sections as thin as possible. Place them in succession upon a slide, with the upper side uppermost, and keep them moistened with alcohol for a few minutes; finally, mount in chloral hydrate.

The outer (upper) part consists of an indistinct tissue in which yeast cells, small crystals, and the hyphæ of fungi can be detected. Next to this is a layer of long narrow cells with

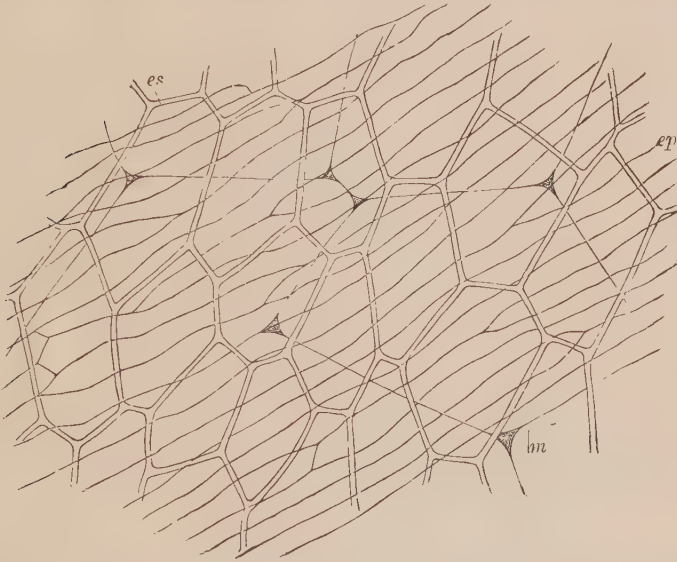


FIG. 113. — Surface view of the epidermis of pericarp and of the seed coats, together with the subjacent mucilage cells. *ep*, epidermis of pericarp; *es*, epidermis of seed coat; *m*, mucilage. $\times 200$. (Tschirch.)

pointed ends, the inner epidermis of pericarp (fig. 113, *ep*); this tissue is usually easily found. Abutting upon this is the outer epidermis of the outer seed coat (fig. 113, *es*); in surface view the cells are large, polygonal, elongated, and thin-walled; they cross the layer above them, sometimes at right angles but more often diagonally. The walls of the mucilage cells which follow next are not easily seen, but there are numerous translucent masses of mucilage.

The nature of the parenchyma that constitutes the bulk of the seed coats is not very readily made out from sections, but

the inner epidermis of the outer seed coat is generally distinct. In surface sections the cells appear small, elongated, polygonal (about $10\ \mu$ by $20\ \mu$), and thick-walled.

Separation by Digestion.—Next proceed to disintegrate the tissues of the shells. Digest a few fragments with a 1 per cent. solution of caustic potash on the water-bath for ten or fifteen minutes (longer if necessary). Tease out a small fragment on a slide with the dissecting needles, and separate the different layers by pressing the fragments firmly with the flat handle of a scalpel. Examine in water or dilute glycerin.

The most conspicuous feature is the presence of numbers of large rounded parenchymatous cells varying mostly from 50 to $100\ \mu$ in diameter. The walls are usually of a pale reddish-brown colour, and exhibit short protuberances, by which they were attached to neighbouring cells; these protuberances seen from above appear as distinct circles. These cells are derived from the parenchymatous tissue of the shell.

Portions of the outer epidermis of the outer seed coat are also easily found. The cells adhere to form large plates; they are large, polygonal, elongated, and have dark brown straight or slightly curved walls. This tissue is often crossed by the long narrow cells of the inner epidermis of the pericarp, the two tissues generally remaining firmly adherent.

The sclerenchymatous cells of the inner epidermis of the seed coat may also be found, but they are usually much less conspicuous than those hitherto described. After the treatment with potash the lumen appears traversed by one or two narrow bars, probably folds of the radial walls.

Lastly numerous spiral vessels from 10 to $20\ \mu$ wide can be found, and also the *débris* of tubular cells from the tissue of the pericarp.

Examination of Powdered Shells

Prepare some powder from the shells, and examine it as follows:

- (1) In water; observe the numerous yellowish or brown masses, in which little structure is discernible, and masses of transparent mucilage usually tinged with brown. Mounted in solution of ruthenium red even the smallest particles of mucilage stain brilliant pink or bright red.

- (2) After warming in chloral hydrate the structure becomes clearer ; portions of the sclerenchymatous inner epidermis, of the outer epidermis of the seed coat, and of the inner epidermis of the pericarp can be detected. The parenchyma can be identified as well as *débris* from the tissue of the pericarp. There are very numerous fragments of spiral vessels.
- (3) Warm a little with solution of potash and endeavour to disintegrate the tissues by pressing upon and moving the coverslip. This preparation usually allows of easy separation of the various layers and their identification.

Diagnostic Characters.—The following are the chief diagnostic characters of the powdered shells :

- (a) The two epidermises ; long narrow cells crossing larger polygonal ones, often diagonally ;
- (b) The small polygonal thick-walled cells of the sclerenchymatous layer ;
- (c) The large rounded parenchymatous cells with arm-like projections ;
- (d) The mucilage.

Examination of Powdered Cocoa

Having thus thoroughly examined the tissues and cell contents of the kernel and the shell, proceed to examine the powder as follows :

(1) Moisten a little thoroughly with water by continued stirring ; mount, and examine in water. There are numerous isolated starch grains, the characters of which can be best examined in water. There are also numerous small and larger masses of tissue in which occasional dark brown cells can be seen, but little that is definite. These dark brown cells turn crimson-red with concentrated sulphuric acid.

(2) Heat the preparation to the boiling-point ; numberless globules of oil separate. The starch, however, gelatinises with difficulty, and even after boiling only the centre of the grains is translucent. The tissues are clearer, and fragments of the red-brown epidermis, of the cotyledons, and also here and there of the endosperm can be detected. The tissues are, however, not yet clear enough for satisfactory examination.

(3) Defat some powder by shaking it with ether-alcohol for a few hours ; wash with alcohol, mount in water. The tissues are clearer than they were in water ; add chloral iodine, they become still clearer and the starch colours blue. Boil gently ;

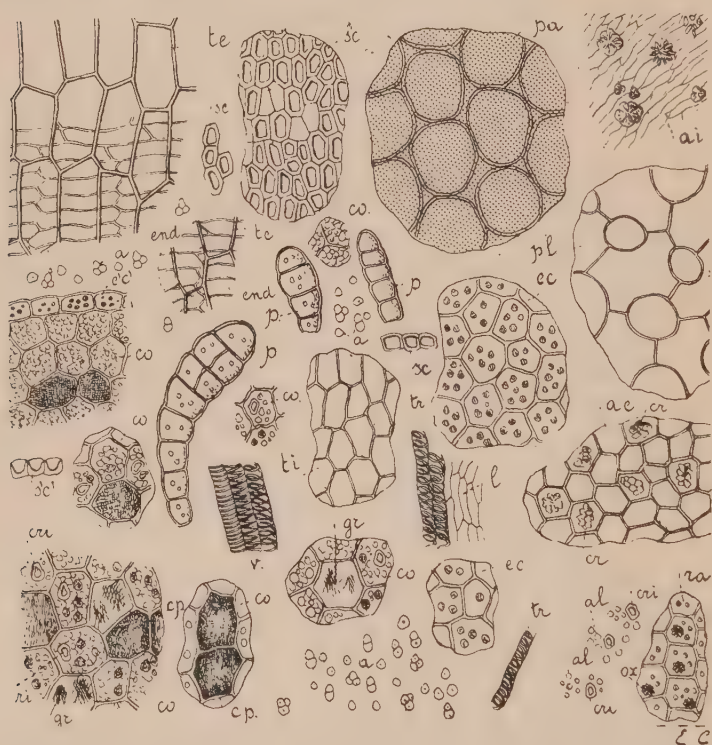


FIG. 114.—Powdered Cocoa. *a*, starch grains ; *ae*, outer layer of endosperm ; *ai*, inner layer of endosperm ; *al*, aleurone grains ; *co*, cotyledon ; *cp*, pigment cells containing cocoa-red ; *cr*, crystals of fat ; *ec*, epidermis of cotyledon, surface view ; *ec'*, epidermis of cotyledon, profile ; *end*, inner epidermis of pericarp ; *gr*, crystals of fat ; *l*, bast from fibrovascular bundles ; *ox*, calcium oxalate crystals ; *p*, pluricellular hairs ; *pa*, *pl*, parenchyma of seed coat ; *ra*, cells of radicle ; *sc*, sclerenchymatous layer of seed coat, surface view ; *sc'*, sclerenchymatous layer of seed coat, in profile ; *te*, outer epidermis of seed coat to which the inner epidermis of the pericarp (*end*) is adhering ; *ti*, inner epidermis of seed coat ; *tr*, *v*, vessels, &c., from fibrovascular bundle.

they now become quite clear. The delicate colourless cell walls of the cotyledons, the pale to dark brown epidermis, and the small crystals of calcium oxalate are easily observed. There are occasional, but not numerous, fragments of fibro-

vascular bundles, but only few of the characteristic hairs are to be found.

Diagnostic Characters.—The chief diagnostic characters of the powdered kernels are :

- (a) The thin-walled parenchyma of the cotyledons.
- (b) The minute starch grains, either simple and rounded, or compound with two or three component grains ; they are difficult to gelatinise.
- (c) The polygonal epidermis of the cotyledons, with dark reddish-brown granular contents.
- (d) The characteristic hairs, which, however, are sometimes rare.
- (e) The abundance of fat.
- (f) The cells containing red-brown cocoa red.

Coffee Beans

Source.—The seed of *Coffea arabica*, Linn.

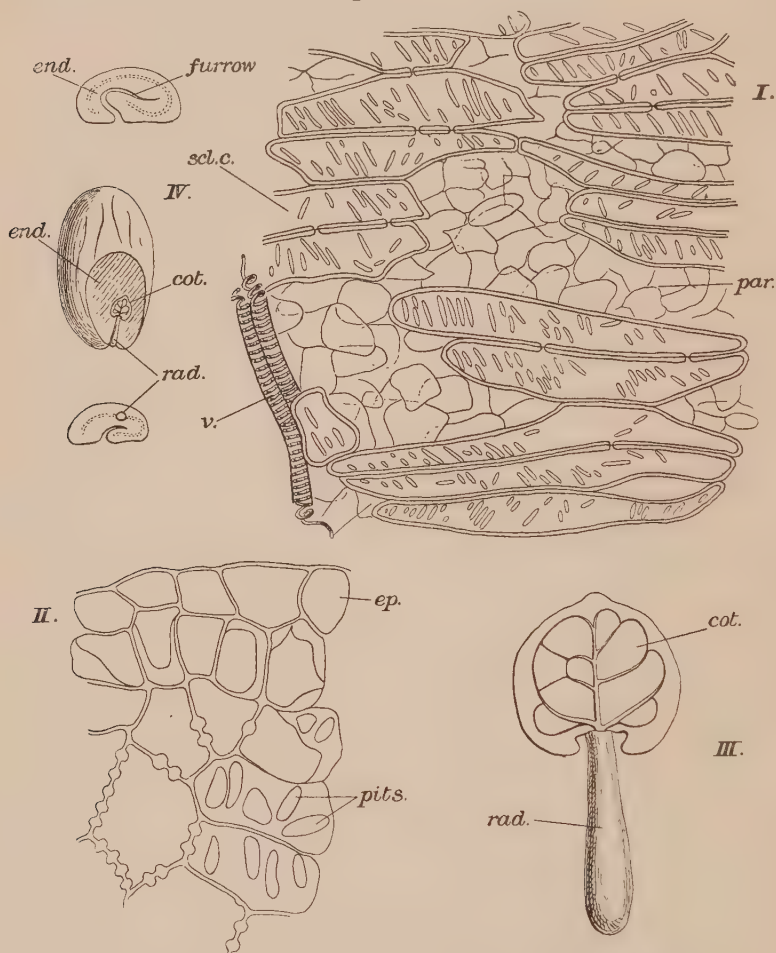
The commercial coffee bean consists chiefly of the endosperm of the seed, the seed coats having been removed by the preparation the beans undergo. Small fragments, however, of the seed coats may be found in the groove running along the flat side of the bean.

Examination of the Seed Coats.—Soften some raw coffee beans by soaking in water for several hours, or in a mixture of equal parts of alcohol and glycerin for twenty-four to forty-eight hours.

Cut one longitudinally through the furrow on the flat side ; from the sides of the groove thus opened strip a small piece of the silvery seed coat. Mount in dilute glycerin or in chloral hydrate.

It is composed of several layers of delicate collapsed parenchymatous cells, but the structure is not very easily seen, a cell wall being visible here and there. In this tissue there are numerous sclerenchymatous cells. Examine these carefully, as they are very characteristic of coffee and always found in it. They are mostly about eight times as long as they are broad ; sometimes they are much broader in proportion than this. They vary exceedingly in size, but many range from 150 to 350 μ in length. They usually taper bluntly, but sometimes they are terminated by flat transverse walls. They are often

arranged side by side with their long axes parallel, and bear numerous large oblique pits. The phloroglucin reaction shows that their walls are lignified.



c. 115.—Coffee. *I.*, portion of the seed coats, surface view; *par.*, collapsed parenchymatous tissue; *scl. c.*, sclerenchymatous cells; *v.*, vessel, $\times 220$; *II.*, transverse section of outer part of endosperm; *ep.*, epidermis, $\times 220$; *III.*, embryo of seed; *cot.*, cotyledon; *rad.*, radicle; *IV.*, sections of seed; *cot.*, cotyledon; *end.*, endosperm; *rad.*, radicle.

Occasional fragments of small vessels derived from the raphe may also be found.

Examination of the Endosperm.—Cut transverse sections, and mount them in chloral hydrate. The epidermis and usually

one or two layers immediately beneath it are composed of cells with evenly thickened walls ; the rest of the endosperm consists of parenchymatous cells with thick walls and very large pits. The pits are so large that they form ovate spaces that may be as long as the cell is wide, or may not attain half that length. In section the cell wall appears thick and beaded. The embryo can be easily dissected out from the soaked berry. The thick radicle and two small cordate leafy cotyledons consist of small delicate parenchymatous cells.

The chief cell contents are oil, proteid matter, and occasional small starch grains.

Examination of Ground Roasted Coffee

The tissues that have been observed suffer no material change by the process of roasting to which the beans are subjected in the preparation of coffee for table use, but the cell contents are partly altered.

Reduce a few roasted beans to a coarsely granular powder, such as coffee commonly forms. Separate the fine particles by sifting ; from some of the coarse ones prepare sections by softening them and fixing them in pith. The sections are very deeply coloured, but may be readily decolourised by a short maceration in solution of chlorinated soda ; examine them in dilute glycerin, and compare with the sections of the bean.

The fine powder is also so deeply coloured as to require some preparation before examination.

Macerate a little in solution of chlorinated soda, separate by centrifugation or otherwise, and wash once with distilled water ; examine in dilute glycerin. The cells of the endosperm are now very clear, and easily examined. The sclerenchymatous cells of the seed coat are also readily found ; these are always present in genuine ground coffee. In the endosperm cells oil globules can be detected.

Digestion with caustic potash may also be employed to remove the colouring matter, but it is not so successful as chlorinated soda.

Ground coffee consists principally of fragments of the endosperm, which have the characters above described. With these there are always associated portions of the seed coat, which are characterised by the sclerenchymatous cells. The

débris of the embryo are not easily found, and there are only very few small spiral vessels present.

Examination of Commercial Coffee

Separate some of the coarsest fragments by sifting ; examine them with a lens, and pick out any that are of suspicious appearance ; soften these, and prepare sections from them ; decolourise, if necessary, with chlorinated soda, and examine.

Decolourise the fine powder that passes through the sieve ; wash, and examine in dilute glycerin. Any foreign fragments are easily detected. For the characters of chicory see page 298.

Reduce a little of the sample to a moderately fine powder and examine this in the same way ; no tissues foreign to genuine coffee should be present.

Grey Pea

Source.—The dried seed of *Pisum sativum*, Linn.

Preparation and Examination of Sections.—Prepare the seeds by soaking in water for twenty-four to forty-eight hours. Should the seed coats separate from the kernel, examine each separately. Embed in pith, and cut transverse sections. Examine them in chloral hydrate. The following tissues can be observed.

Epidermis, formed of palisade cells about 12μ wide and 58μ or more high. The walls are cellulose, and are strongly and irregularly thickened. The lumen of the cell narrows near the centre, and expands again towards the base into a cavity with an irregular wavy or jagged outline. The walls are traversed by pits, which appear in this section as longitudinal striations ; they are better seen in a surface preparation.

Hypoderma, consisting of a single layer of cells about 30μ wide and the same height. These cells are constricted about the middle, and thus assume the shape of a dumb-bell or capstan. They are known as 'bearer' cells. Their walls are rather thick and not lignified ; they are traversed by longitudinal pits, which often widen as the cell widens.

Parenchyma, composed of six or eight rows of delicate more or less collapsed parenchymatous cells of moderate size.

If the seed is examined carefully there will be seen near the hilum a small lenticular patch of a whitish colour. Transverse sections through this patch show that here the epidermis

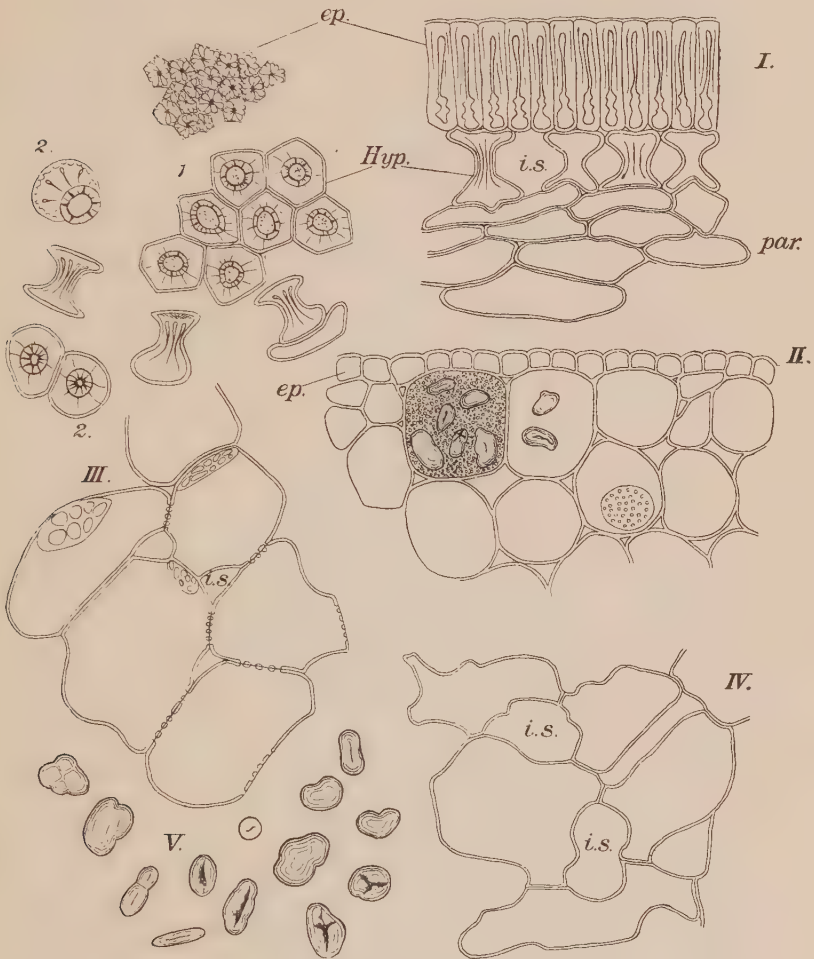


FIG. 116.—Pea. I., transverse section of seed coat; *ep.*, epidermis; *Hyp.*, hypodermis; *i.s.*, intercellular space; *par.*, parenchyma; 1, cells of hypodermis viewed from below; 2, viewed from above and from side. II., transverse section of outer portion of cotyledon; *ep.*, epidermis. III. and IV., parenchyma of seed coat, surface view. V., starch. $\times 250$.

consists of a double row of palisade cells, and also that it is not continuous, but exhibits a narrow slit which runs along the oval patch, opening towards the seed coats. Below this slit

and parallel to it there is a collection of lignified porous cells, which in transverse section is flask-shaped. These cells are regarded as tracheids, but their function is not accurately known; they are found in nearly all leguminous seeds. The tracheids are surrounded by three rows of delicate parenchymatous cells.

The presence of these tissues necessitates a considerable increase in the thickness of the seed coats in their neighbourhood. The hypoderma is replaced by about three rows of thick-walled deeply pitted cells resembling ordinary sclerenchymatous cells, which pass into rounded thick-walled cells with short projecting arms, forming a loose tissue with numerous air spaces.

Surface Preparations.—Separate the seed coats and macerate for twelve hours in cold 5 per cent. solution of potash; tease well out with the needles.

Epidermis; the cells are polygonal in surface view, and exhibit numerous pits, which appear as dark lines radiating from a small dark point (cell lumen).

Hypoderma; the cells are polygonal in outline and have pitted walls; on focussing down the constricted portion of the cell appears as a bright ring in the centre of the cell; this ring is traversed by pits, and similar pits extend from the margin of the ring to the outer line. This peculiarity is easily explained by a comparison with the section of the cell.

Parenchyma.—The cells are thin-walled, and exhibit intercellular spaces; near the cotyledons they are of considerable size.

Examination of the Cotyledons.—Proceed now to examine the cotyledons. Prepare transverse sections from a soaked seed, and examine in dilute glycerin.

The epidermis consists of small cells which are square in transverse section, and contain aleurone grains but no starch. Below the epidermis are large rounded parenchymatous cells, with small intercellular spaces and pitted walls. They contain starch grains and innumerable small aleurone grains. In surface view the epidermal cells are seen to be narrow and elongated.

Examination of the Starch.—Examine some of the starch grains in water. They vary somewhat in shape, but many are oval-oblong, subreniform, or rounded, the majority showing remarkable enlargements at different points. Length about 20 to 40 μ .

Examination of the Powder.—Mount in water, and examine the starch grains.

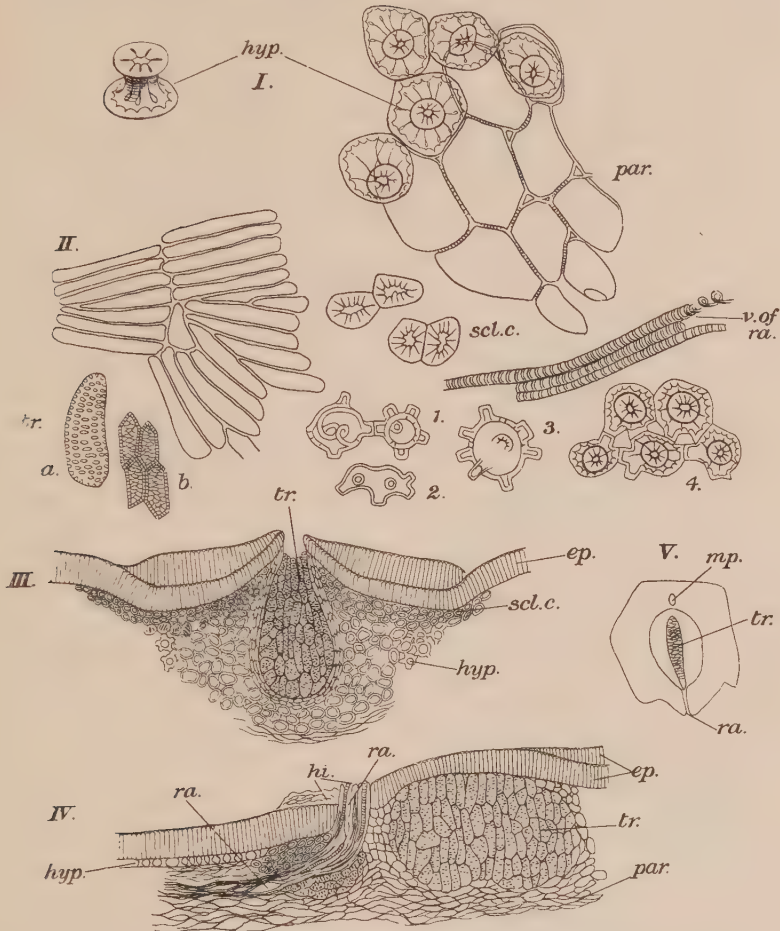


FIG. 117.—Pea. I., hypodermis viewed from above, with subjacent parenchyma. II., epidermis of cotyledon, surface view; 1, 2, 3, 4, modified epidermal cells from near the furrow. III., transverse section through the group of tracheids; *ep.*, epidermis; *hyp.*, modified hypodermis; *scl. c.*, sclerenchymatous cells of same; *tr.*, tracheids. IV., longitudinal section through the group of tracheids; *ep.*, epidermis; *hi.*, hilum; *hyp.*, hypodermis; *par.*, parenchyma; *ra.*, raphe; *tr.*, tracheids. V., lens view of group of tracheids. I. and II., $\times 250$; III. and IV., $\times 50$.

Mount in chloral hydrate, warm, and run in glycerin. The cells of the cotyledons are distinguishable everywhere. The epidermis is often in flakes, exhibiting its surface view. The

hypoderma is also easily found, and, like the epidermis, exhibits its surface view, the cells being easily identified. The sub-jacent parenchyma can also be detected, but it is not shown

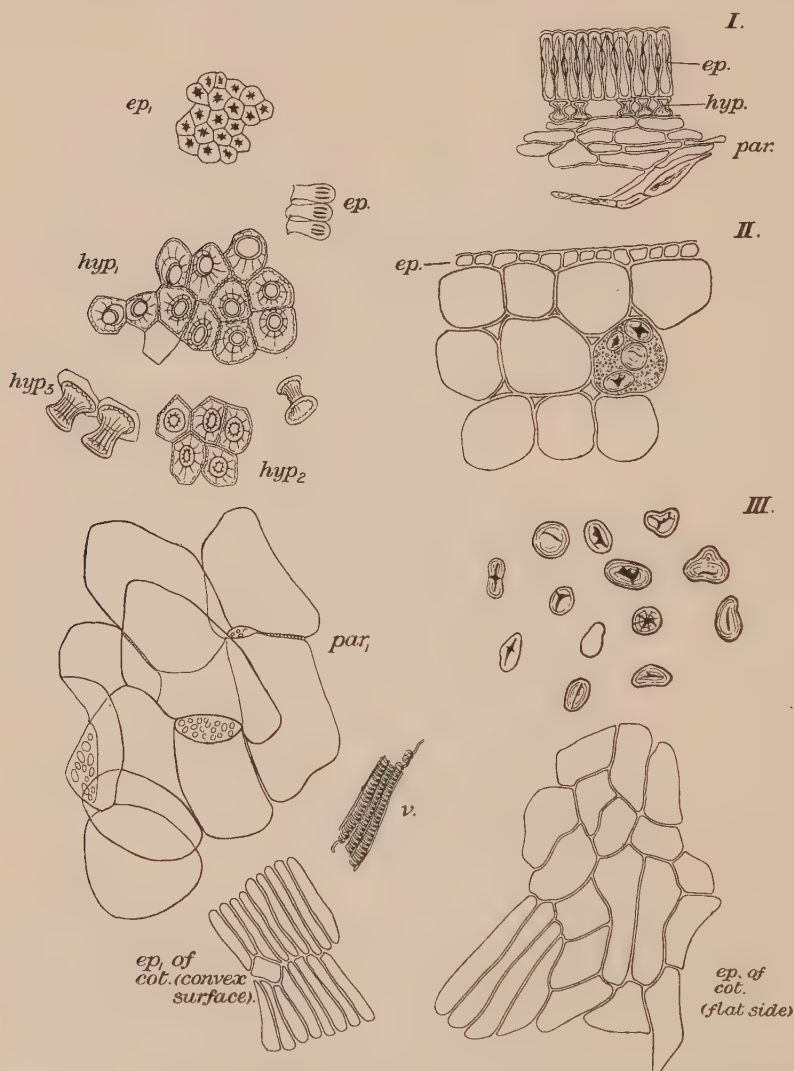


FIG. 118.—Lentil. I., transverse section of seed coat; *ep.*, epidermis; *hyp.*, hypoderma; *par.*, parenchyma. II., transverse section of cotyledon; *ep.*, epidermis. III., starch grains; *ep.*, epidermal cells, after treatment with potash; *ep.*, epidermal cells, surface view; *hyp1*, hypoderma viewed from below; *hyp2*, the same from above; *hyp3*, hypodermal cells isolated by potash; *par1*, parenchyma of seed coat, surface view; *v.*, vessels from raphe. All $\times 200$.

very clearly. Here and there spiral vessels from the raphe may be found, but the tracheids from the neighbourhood of the hilum require diligent searching before they can be detected.

The structure of the pea may be taken as typical of

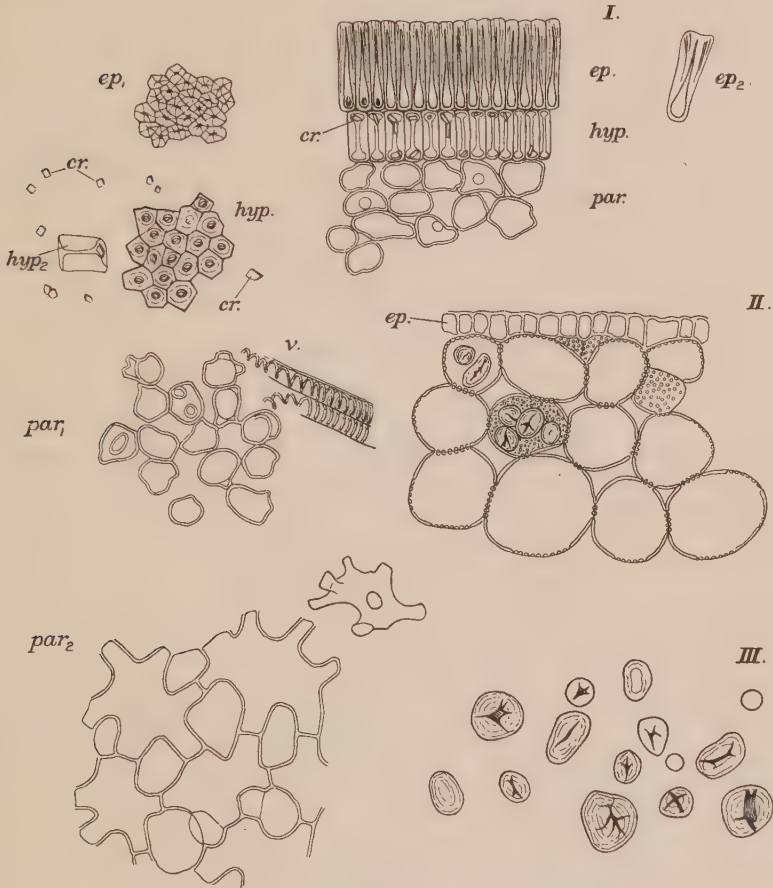


FIG. 119.—Haricot Bean. *I.*, transverse section of seed coat; *cr.*, crystals of calcium oxalate; *hyp.*, hypodermis; *par.*, parenchyma. *II.*, transverse section of cotyledon; *ep.*, epidermis. *III.*, starch grains; *ep.*, epidermis of seed coat, in surface view; *ep.*, epidermal cell, isolated by potash; *hyp.*, hypodermis, in surface view; *hyp.*, hypodermal cell, isolated by potash; *par.*, parenchyma of seed coat, surface view; *par.*, inner layers of same. All $\times 200$.

leguminous seeds. These are characterised by the remarkable palisade epidermis, by the bearer cells, and by the group of tracheids near the hilum. Most of them also contain starch grains more or less closely resembling those of the pea or bean

in general features. Leguminous flours are, therefore, easy of detection when mixed with cereal or other flours. Vogl¹ gives the following useful table showing the chief points of distinction between the various leguminous flours. In order to facilitate comparison, illustrations of the structure of the lentil and haricot bean are appended.

	BEAN	PEA	LENTIL
(a) Palisade epidermis	30 to 60 μ long; not conical towards cuticle; wall smooth on interior; lumen wide near the inner wall, gradually or quickly contracting towards the outer wall	75 to 110 μ long (mostly 90 μ), not conical towards cuticle; inner wall irregular; lumen wide near the inner wall, contracting in the middle and widening again near the exterior	Up to 45 μ long, with a short conical projection towards the cuticle; inner wall smooth; lumen very wide, contracting towards the outer wall
(b) Hypodermis	Transverse section four-sided, without intercellular spaces (radial diameter 15 to 30 μ , transverse 15 to 25 μ), thickened at the sides and containing crystals of calcium oxalate (6 μ)	Delicate goblet, beaker, or dumb-bell shaped cells, inner wall rather broader than the outer; at the side large intercellular spaces (radial diameter 30 to 36 μ , transverse 36 to 45 μ), rather thick-walled, with cleft pores, but without crystals	Compressed dumb-bell or hourglass shaped, seldom elongated, often irregular, with intercellular spaces and cleft pores (radial diameter 9 to 24 μ , most 15 to 18 μ ; transverse 15 to 30 μ), without crystals
(c) Parenchyma of cotyledons	Cells with thick walls and large pores, wall at least 5 μ thick and coarsely beaded in transverse section	Cells with moderately thick walls, pitted; wall at most 3 μ thick; cell wall in transverse section smooth or slightly beaded	Cells with thin walls, which are only slightly or indistinctly pitted in transverse section
(d) Starch	Grains up to 57 μ , the majority of regular elliptical, reniform, or bean-shaped, with long branching cleft through the hilum and very distinct striations	Grains 15 to 47 μ (or up to 51 μ), principally grains of irregular shape with rounded protuberances, together with reniform and bean-shaped grains, few being regularly elliptical; many without a fissure through the hilum; hilum and concentric striation visible	Grains 9 to 45 μ (most 20 to 40 μ) partly resembling bean and partly pea starch; many show concentric striations, but not so distinct as bean starch; many have no fissure, others a small unbranched fissure through the hilum

¹ *Nahrungs- und Genussmittel*, p. 166.

Identification of a Powder as that of a Seed

Among the characters that distinguish powdered seeds from other powders the aleurone grains occupy a position of great importance. These grains are found in ripe seeds only, and, if found, point definitely to the presence of a seed. Large and well-developed aleurone grains are comparatively easy to detect and examine, but that is by no means the case with those of very small size.

The presence of much reserve starch, fixed oil, or fat also indicates a seed powder, and the same may be said of reserve cellulose, which generally occurs in the form of strongly thickened walls of the endosperm.

Notable quantities of vascular tissue are usually absent, the only vessels found in seed powders being those derived from the raphe, and these are commonly very small. Sclerenchymatous fibres (bast fibres) are also absent, and the powder should be free from chlorophyll, from epidermis with stomata, and from cork tissue.

SECTION X

FRUITS

INTRODUCTION

FRUIT is the term applied by botanists to the whole product of the development of the gynœceum as a result of fertilisation. Sometimes other parts of the flower in addition to the gynœceum participate in the production of the fruit, which is then termed spurious.

In dealing, therefore, with the anatomy of the fruits, it is evident that in addition to such tissues as form the seed coats and kernel of the seed the structure of the tissues that have been developed from the carpellary walls of the ovary must be studied, and also, if the fruit examined be a spurious one, the structure of the tissues that are derived from such other parts of the flower as combine to produce the organ under examination.

The seeds that are developed in dehiscent fruits have as a rule to remain for some time exposed to the inclemencies of the weather, and in such seeds it is natural to expect a considerable development of the seed coats into more or less resistant, protective coverings. The seeds described in the preceding section were seeds of dehiscent fruits, and, as a general rule, it will be found desirable with such fruits to remove the seeds from the pericarp and examine each separately. But the seeds contained in indehiscent fruits usually rely more or less completely upon the protection afforded to them by the tissues of the enveloping pericarp, and in such cases it is frequently found that the seed coats are reduced to a very narrow layer composed of but few rows of cells which have often so completely collapsed as to render their structure difficult of examination. Pepper and cubebs, the umbelliferous fruits, and many others furnish examples of this. So also do the Gramineous fruits, which,

from the large extent to which they serve as food stuffs, are especially important. Among the latter barley may particularly be noticed, inasmuch as, in addition to the pericarp and seed coats, the tissues of the paleæ, which remain permanently attached to the pericarp and hence form part of the so-called fruit, have to be considered; indeed, as far as its structure is concerned, this fruit is one of the most complex with which the histologist has to deal.

The great diversity in structure exhibited by the official fruits results in the presentation of a number of diagnostic characters that may be utilised in the identification of the drug or its powder, but it also brings with it the absence of any general structural plan such as that observed in leaves, stems, and barks.

The epidermis is in most cases well preserved, and the form and disposition of its cells may be useful. Stomata are generally recognisable, though they have often undergone considerable change. Like the leaves, the epidermis of the pericarp may bear hairs, which then offer valuable diagnostic features.

The tissue subjacent to the epidermis is, in many cases, a parenchyma traversed by fibro-vascular bundles and comparable with the mesophyll of the leaf. In this tissue sclerenchymatous cells or groups of cells are often to be found (pepper, pimento, cubeb). In such cases the characters of these cells must be accurately determined. Oil cells (pepper, cubeb), oil glands (pimento), oil ducts (most umbelliferous fruits), laticiferous vessels (poppy capsules)—in short, secretory tissue of various kinds—may be present, all of which are of high importance. Calcium oxalate, starch, and other cell contents contribute their share to the characteristics of the drug.

On the inside the pericarp is bounded by an epidermis which, like the outer epidermis, may present particular features.

Diagnostic characters of fruits may be sought for in the following points (in addition, of course, to those furnished by the seed, which have been dealt with in the preceding section).

- (a) The outer epidermis, more particularly in the hairs, if present.
- (b) The sclerenchymatous tissue in the subjacent parenchyma; the shape of the cells, their size, character of the pits, &c.

- (c) The presence and character of secretory tissue of any kind.
- (d) The cell contents, more particularly calcium oxalate and starch.
- (e) The characters of the inner epidermis.

Cardamom Fruit

Source.—The fruit of *Elettaria Cardamomum*, Maton.

Examination of the Seeds

Preparation.—Procure some Mysore cardamom fruits and separate from them some seeds that are full grown and nearly, but not quite, ripe; these may be recognised by their colour, which is not quite so dark as that of the ripe seeds. The structure is similar, but the seeds are easier to cut before they are quite ripe.

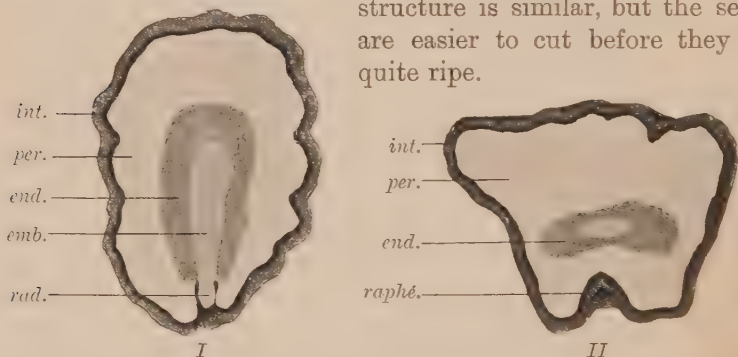


FIG. 120.—Cardamom Fruit. *I.*, longitudinal section of seed; *II.*, transverse section of the same; *emb.*, embryo; *end.*, endosperm; *int.*, integuments; *per.*, perisperm; *rad.*, radicle. $\times 14$.

Soak them in water for several days: should they become too soft, harden them a little in spirit.

After soaking in water a semi-transparent membranous coat becomes visible, which was previously scarcely to be detected: this is the arillus. It is attached to the micropylar extremity of the seed.

Remove the arillus, and cut the seed longitudinally in half. Examine with a lens, and observe in the centre the small elongated-oval embryo, surrounded by a scanty yellowish endosperm, which is itself enclosed in a more abundant whitish perisperm, the whole being surrounded by a dark narrow seed

coat. Cut another seed transversely, and identify the same parts in transverse section.

Arillus.—Soak a seed for a few minutes in water, and strip the arillus from it; mount, and examine in water or dilute

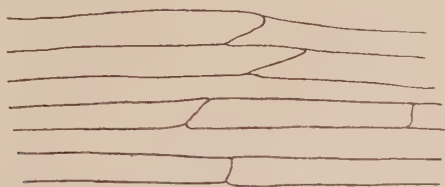


FIG. 121.—Cardamom Fruit. Arillus of seed. $\times 200$. (The cell contents are not shown.)

glycerin. It is composed of several layers of narrow elongated cells, with delicate walls, which are often not very easy to see; they contain minute globules of oil and occasional small rosettes of calcium oxalate.

Sections of Seed Coats.—Fix a seed in pith (or cork) and cut transverse sections; examine in water or dilute glycerin.

All the cells of the perisperm are filled with dense granular masses, which on closer examination prove to be minute rounded or angular starch grains about 2 or 3 μ in diameter. In most of the cells there is also a larger body visible, usually in a small cavity near the centre; in some of the cells this can be identified as a prismatic crystal of calcium oxalate, but it can be better examined after gelatinisation of the starch.

The cells of the endosperm and embryo contain hyaline masses, which will be subsequently more closely examined.

Mount another section in solution of potash; warm gently until the starch is gelatinised, but do not boil.

The outermost layer of cells is the epidermis of the seed

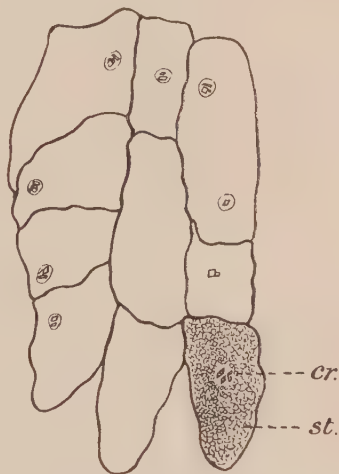


FIG. 122.—Cardamom Fruit, portion of perisperm. *cr.*, crystals of calcium oxalate; *st.*, starch (represented in one cell only). $\times 240$.

coat. The cells appear small and nearly quadratic in section. In the depressions of the seed they are larger than they are in the elevated parts.

Examine a section (not previously warmed with potash) in glycerin. The cells are thickened on the outer and inner walls; it is evident on comparison that warming with potash has made them swell.

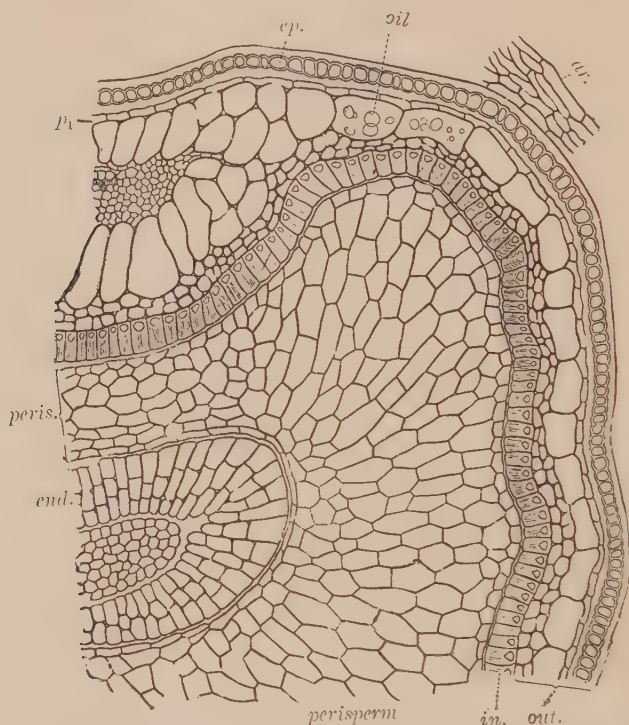


FIG. 123.—Cardamom Fruit, transverse section of seed. *ar.*, arillus; *end.*, endosperm, enclosing the embryo; *ep.*, epidermis; *in.*, inner integument; *oil*, oil cells with globules of oil; *out.*, outer integument; *p₁*, subepidermal parenchyma. (After Tschirch and Oesterle.)

Next to the epidermis observe, in the potash preparation, very flat collapsed cells often coloured brown by the action of the alkali (fig. 123, *p₁*); these separate the epidermis from a single row of large rectangular cells with thin strongly refractive walls. The brown cells are often rather difficult to detect, but they may generally be found by carefully examining the line that separates the rectangular cells from the epidermis. They constitute the second layer of the seed coat.

The large rectangular cells just referred to constitute the third layer. They contain volatile oil, but it is not often that the oil can be seen in them, especially if the sections are thin. Its presence can, however, be easily demonstrated (see below).

Following upon the oil cells is a confused narrow layer of tissue which may be shown by the examination of earlier stages of development to be the remains of a single or double layer of cells, but its structure cannot always be distinguished in a transverse section. This is the fourth layer.

Next to the collapsed layer is a very conspicuous single row of brown or yellowish-brown cells. They are rectangular and radially elongated; about 40 μ long and 20 μ wide. The radial and inner tangential walls are so strongly thickened that the lumen appears small and funnel-shaped, the mouth of the funnel being directed outwards. The walls appear faintly striated. This, the fifth layer, is the inner integument of the seed, and within it is the perisperm, in the cells of which the calcium oxalate crystals are now distinctly visible; sometimes there is only one crystal in each cell, but sometimes there are three or four.

Take another section and boil very gently in solution of potash for a few seconds. Examine the sclerenchymatous cells of the inner integument, and observe in the lumen of each cell, nearly filling it, is a small rounded or subconical body with a granular surface. These have been carefully examined and tested in various ways; they appear to be nodules of silica.

Warm another section in chloral hydrate, cool, and examine; the calcium oxalate crystals in the cells of the perisperm are very distinct.

Surface Preparations of Seed Coats.—Prepare surface sections from a seed; the sections are apt to be difficult of interpretation, owing to the rugose surface of the seed. Examine them in succession, mounting in chloral hydrate, and paying, as usual, particular attention to the order in which the various layers of cells are arranged. Care must be taken to keep the outer surface uppermost.

The epidermis is easily identified by its very long narrow cells; the width of the cells should also correspond to the width of the epidermal cells seen in the transverse section. The oil cells (third layer) are conspicuous by reason of their large size and thin highly refractive walls, but the cells of

layer 2, which lies between the epidermis and the oil cells, are inconspicuous. They can, however, with care also be distinguished: their long axes cross the long axes of the epidermal cells at right angles.

The layer of collapsed cells which follows next is very difficult to distinguish in surface preparations, but the sclerenchymatous cells are again easy of observation. The latter layer varies in colour from brownish-yellow to dark brown, according to the degree of ripeness of the seed. In surface view the cells are small (about 15 to 25 μ), polygonal, and exhibit small cavities: their appearance, therefore, is quite different from that exhibited by the transverse section.

Within the sclerenchymatous layer the thin-walled cells of the perisperm, crowded with minute starch grains, occur.

Maceration Preparations of Seed Coats.—Prepare next maceration preparations of seed coats.

Digest a few seeds for an hour in a water-bath with 5 per cent. solution of potash; wash with distilled water; tease out, and break up the seed coats as thoroughly as possible.

The epidermis is generally easily found (fig. 124, *ep*); it occurs mostly in flakes, but some of the cells will probably have separated from one another. Here and there delicate thin-walled cells can be seen; they are much shorter, but as wide as or rather wider than the epidermal cells, which they cross at right angles. These are the cells of layer 2, and it is in this preparation that they can best be seen (fig. 124, *par*₁).

The oil cells are very conspicuous; they are large thin-walled cells of rectangular shape, without intercellular spaces; each one contains a large globule of oil.

Layer 4, which follows upon the oil cells, is with difficulty to be found in the potash preparation.

The sclerenchymatous layer is very much darkened in colour by the action of the alkali, so that this layer from ripe seeds is almost black, and shows little or no structure: from unripe seeds it is reddish-brown, and the nodules of silica can be seen if the light is sufficiently powerful. Layer 4 often adheres to this sclerenchymatous layer, and can best be found by separating the fragments of the latter and breaking them up as completely as possible. The cells of which layer 4 consists are rounded or polygonal, nearly isodiametric, and have thin delicate walls.

Sections of the Kernel.—Having now thoroughly examined the seed coats, the student may proceed to the kernel, consisting of perisperm, endosperm, and embryo.

Cut a few transverse sections and examine in water.

The cells of the perisperm are packed with minute starch grains; in the centre of each cell there is a cavity in which one or more minute crystals of calcium oxalate may be seen.

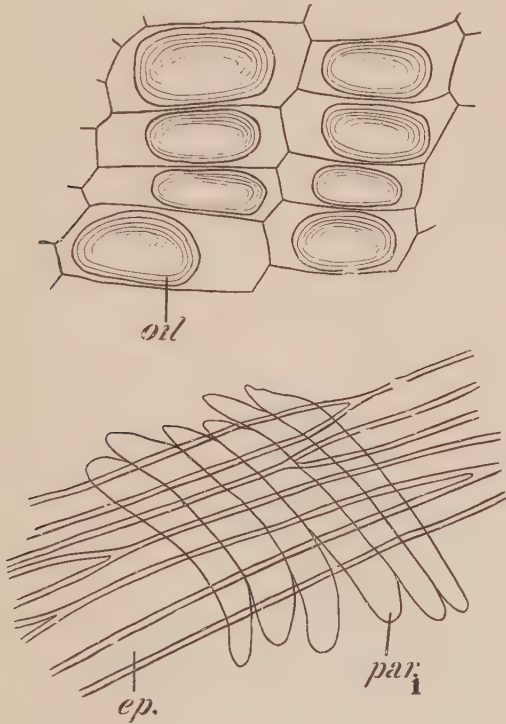


FIG. 124.—Cardamom Fruit, elements of the seed. *ep.*, epidermis; *par_i*, subjacent parenchyma; *oil*, oil cells; isolated by maceration with potash. $\times 240$.

Examine the starch grains; they are very small (1 to 3 μ), rounded or angular, and often translucent in the centre.

Next mount one or two sections in chloral hydrate, and warm until the starch is gelatinised, cool, add a drop of glycerin, and examine.

The walls of the cells can be distinctly seen; they are thin and often wavy; the calcium oxalate crystals are now very distinct.

The endosperm and embryo are best examined as follows :

Mount a transverse section of the seed in chloral iodine ; observe that the starch contained in the cells of the perisperm stains blue, but the contents of the endosperm assume a distinct yellow colour, those of the embryo becoming less deeply coloured.

Mount another section in chlorzinciodine ; the very delicate cell walls of the endosperm can be seen, if carefully examined.

Mount another section in water or chloral hydrate ; oily globules exude from the cells of the embryo. If water is used the globules may be stained in the usual way.

Mount a defatted section in picric acid as directed for mustard seed ; observe the cells of the embryo ; they are small, and filled with small aleurone grains, which stain with picric acid ; the cell contents of the endosperm also stain.

The student will have now thoroughly examined all parts of the seed, and may proceed to identify them in the powder.

Examination of Powdered Seeds

(1) Mount a little powder in water or dilute glycerin as usual, and examine. Very conspicuous are the perisperm cells packed with starch grains, and containing calcium oxalate crystals (better seen after gelatinisation of the starch). Irrigate with dilute solution of iodine ; the starch turns blue.

(2) Mount a little in chloral iodine ; the starch in the cells of the perisperm assumes a blue colour, but the cells of the endosperm and embryo are coloured yellow.

(3) Mount a fresh portion in saturated solution of picric acid as directed for mustard seed ; the fragments of endosperm and embryo are stained deep yellow ; they may be made more conspicuous by warming until the starch in the cells of the perisperm is gelatinised. With care the cell walls can be detected, especially after the addition of a little glycerin.

(4) Mount a little of the powder in Fehling's solution, heat to boiling, and cool ; the fragments of endosperm are coloured violet (Meyer).

(5) Mount a little in chloral hydrate as usual, warm until the liquid boils, cool, and examine. In this preparation there can be found without difficulty—

- (a) *Pieces of the Epidermis*; the epidermal cells are easily recognised by their moderately thick, straight, or slightly curved walls, and more or less pointed ends. They are often crossed at right angles by the cells of the second layer; the latter are shorter and have thinner walls, but are of about the same width.

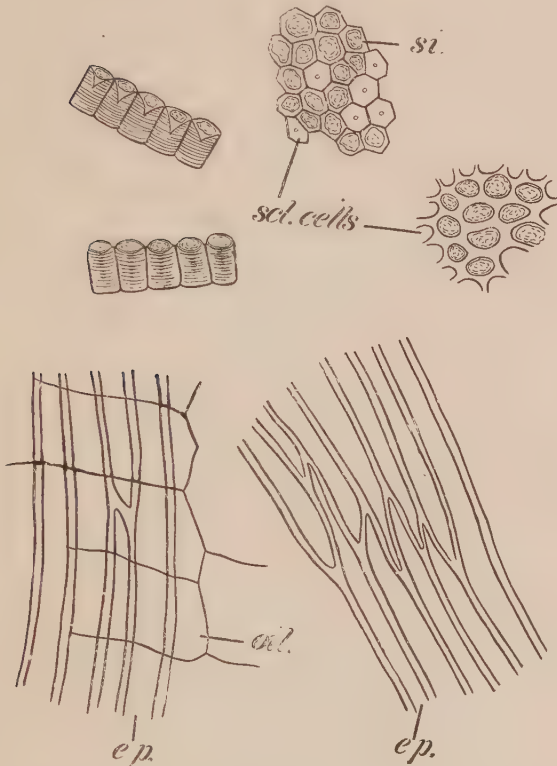


FIG. 125.—Cardamom Fruit. Characteristic elements of the seed coats, from the powder. *ep.*, epidermis; *oil*, oil cells, broken and free from oil; *scl. cells*, sclerenchymatous cells of inner integument; on the left in section; on the right in surface view from below (upper figure) and from above (lower figure); *si.*, nodule of silica. $\times 240$.

- (b) *Fragments of the Sclerenchymatous Layer*; these are very conspicuous by reason of their colour, which varies from yellowish in unripe to dark reddish-brown in ripe seeds. The surface, viewed from above, exhibits rather large cell cavities, in many of which the nodule of silica can be seen, the dark colour beneath

the nodule being due to the thickened and coloured walls of the cell (compare fig. 125). Viewed from below the polygonal outlines of the cells are distinct, and the cavities small; in this aspect the delicate outlines of the layer 4 are often to be distinguished lying over the sclerenchymatous layer.

- (c) *Portions of the Perisperm*; the cells are readily identified by their size, by their moderately thick pitted walls, and by the crystals of calcium oxalate which they contain.

Less easily found and identified are :

- (d) The narrow, elongated, thin-walled cells of the arillus.
 (e) Portions of the endosperm and embryo; the cells have thin walls, not pitted, and do not contain calcium oxalate crystals.
 (f) *Débris* of the oil cells; these are mostly broken, and free from oil.

Examination of the Pericarp

Sections.—Proceed next to the examination of the pericarp. Separate some pericarps from the seeds, and soften them by exposing them to a moist atmosphere for twelve hours. Cut transverse sections, place them for a moment in alcohol, transfer to water, and finally mount in dilute glycerin.

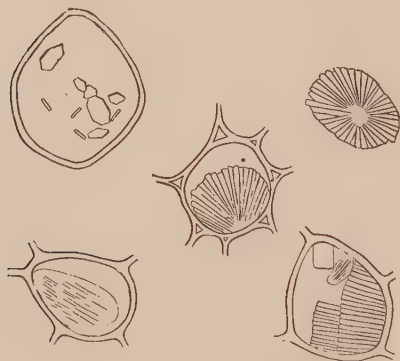


FIG. 126.—Cardamom Fruit. Calcium oxalate crystals in the parenchymatous cells of the pericarp. $\times 240$.

The section consists of parenchymatous tissue traversed by numerous fibro-vascular bundles, and bounded on the outer side by an epidermis consisting of small flattened cells, on the inner side by

an epidermis, the cells of which are often so collapsed as to show little structure. The cells of the parenchyma are large, and have thin walls; most of them are empty, but here and

there irregular sphærocrystalline masses can be detected ; these can be shown by the usual tests to be calcium oxalate. They are rather irregular in distribution, and are best seen in sections taken from the inner surface. Under the polariser they are easily

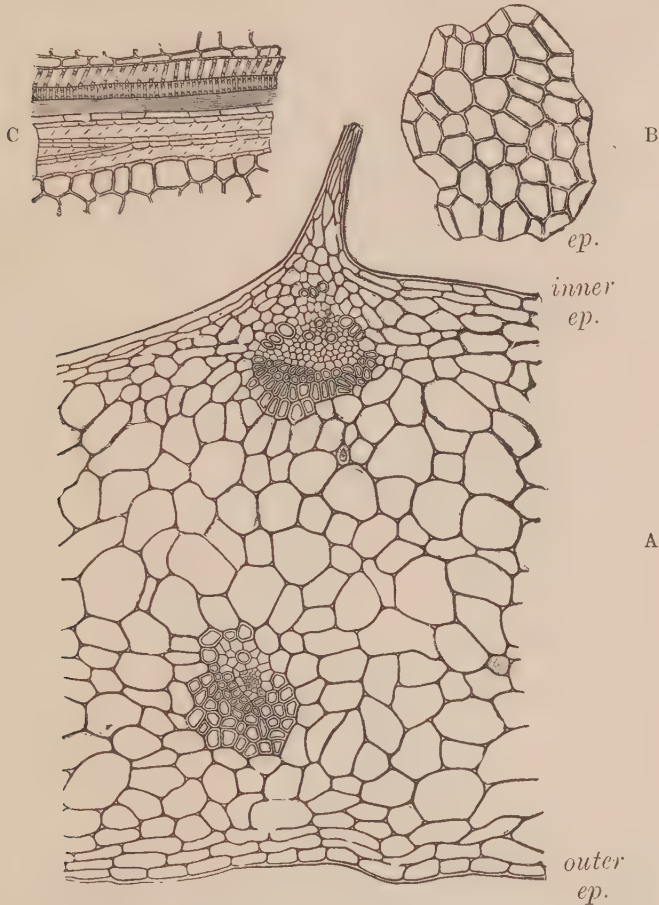


FIG. 127.—Cardamom Fruit. Structure of the pericarp. *A*, transverse section, with projecting carpellary wall, showing two fibro-vascular bundles with supporting sclerenchymatous tissue; *B*, outer epidermis of pericarp, surface view; *C*, longitudinal section of bundle. (Tschirch and Oesterle.)

detected. This tissue also contains scattered rounded or oval cells filled with a yellow or brownish resin.

The fibro-vascular bundles consist of a few small spiral vessels and a scanty bast protected (or surrounded) by an

abundant crescent (or circle) of sclerenchymatous tissue. The elements of the latter have large cavities and not very thick walls.

Maceration Preparations.—Macerate some fragments of the pericarp in potassium chlorate and nitric acid; wash, and tease out the sclerenchymatous cells and fibres. They vary considerably in length, in diameter, and in shape. They average about

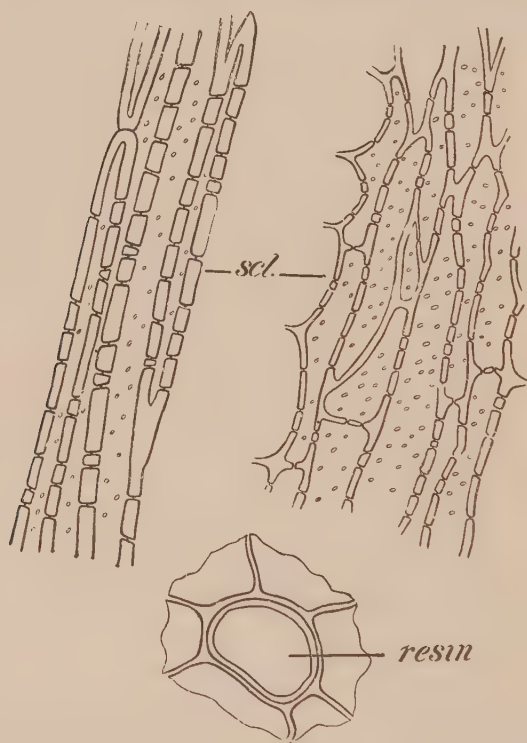


FIG. 128.—Cardamom Fruit. Sclerenchymatous cells and resin cell from the pericarp. $\times 240$.

600 μ in length and 30 μ in width, but may be found more than 1,000 μ or less than 10 μ long. The ends are rounded or blunt, not sharply pointed, and the walls are not very thick. They often have a wavy outline, due to the pressure of neighbouring cells. The pits appear, after treatment with the oxidising mixture, to be slits arranged in a left ascending spiral.

Examination of Powdered Fruit

The following examination should reveal the tissues and cell contents of the pericarp, in addition to those derived from the seed :

- (a) In water or dilute glycerin, large, empty, thin-walled parenchymatous cells, with an occasional cell filled

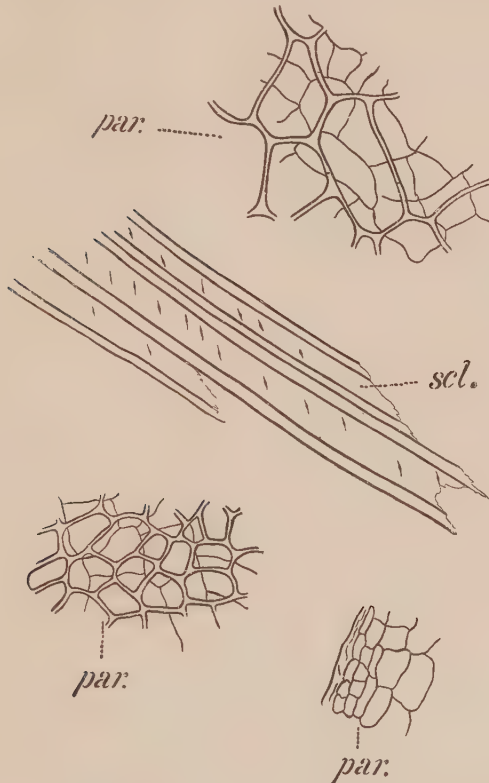


FIG. 129.—Cardamom Fruit, fragments from the powdered pericarps.
par., parenchymatous tissue ; *scl.*, sclerenchymatous fibres. $\times 240$.

with yellow or brown resin ; groups of lignified cells and fibres, which may be distinguished from epidermal cells by the lignification of the walls and by the conspicuous pits.

- (b) In chloral hydrate the fibres are well seen, and the radiating groups of calcium oxalate crystals, characteristic of the pericarp, may also be found.

- (c) Macerate 0.5 gramme of the powdered fruit with 5 c.c. of water, 10 c.c. of nitric acid, and 1 gramme of potassium chlorate on a water-bath until chlorine is evolved and the powder bleached; the starch and other cell contents should be entirely destroyed, the cellulose partly so, but the sclerenchymatous tissue should not be vigorously attacked. Separate the cell *débris* by a centrifuge, wash, and examine in water.

Particularly distinct in this preparation are the nodules of silica from the inner integument of the seed. They appear as small oval or rounded masses with a granular surface. The inner integument itself is also well seen, as the colour is partly discharged and the nodules of silica are very conspicuous. The fibres from the pericarp are very distinct, and often show spiral disintegration.

Colocynth Fruit

Source.—The fruit of *Citrullus Colocynthis*, Schrader. The fruits, which are more or less completely freed from the outer rind, may be divided into the following parts for examination:

- (a) The rind. (b) The pulp. (c) The seed.

Examination of the Rind

Cut from a colocynth fruit one or two of the fragments of rind that are often left adhering to it. Expose them to a moist atmosphere for a few hours, and cut transverse sections; allow these to remain in alcohol as long as possible. Mount one in dilute glycerin or chloral hydrate. The section shows an epidermis consisting of a single row of radially elongated cells. These cells are about $15\ \mu$ wide and $20\text{--}25\ \mu$ high; the radial walls are strongly but not uniformly thickened, being thickest near the middle, tapering slightly towards the outside, but abruptly narrowing towards the inside. The outer tangential wall is cuticularised, and so are the radial walls, with the exception in each case of an inner layer of cellulose lining the cell. In surface sections the cells are polygonal, small, and rather thick-walled. Scattered over the surface of

the fruit are large depressed stomata surrounded by thin-walled cells; the stomata can also be observed in transverse sections.

Following upon the epidermis is a layer about $150\ \mu$ wide, consisting of some fifteen rows of thin-walled, tangentially elongated, parenchymatous cells with pitted walls.

Next to this is a layer of about the same thickness, consisting of several rows of sclerenchymatous cells. Those near the outside are about 15 to $30\ \mu$ in diameter, rounded or radially elongated, nearly isodiametric, and provided with very thick pitted walls. Towards the interior of the layer the cells are

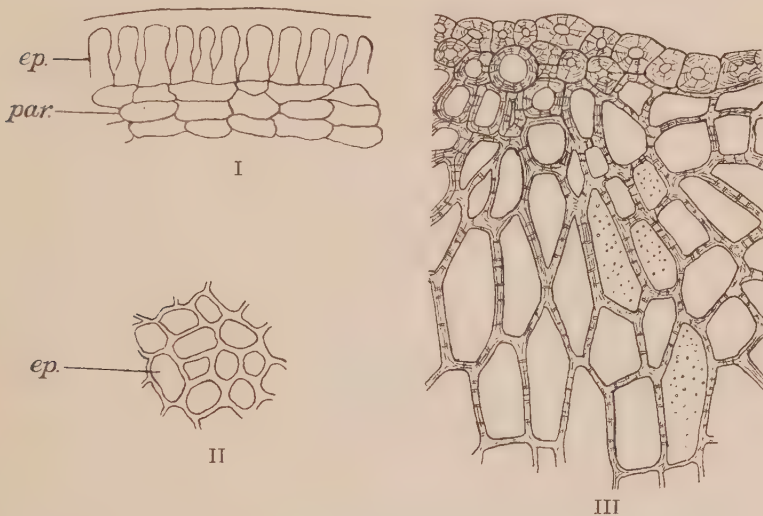


FIG. 130.—Colocynth Fruit. I, transverse section of outer portion of rind; *ep.*, epidermis; *par.*, subjacent parenchyma. II, epidermis in surface view. III, transverse section of sclerenchyma lying below the parenchymatous layer. $\times 240$.

larger (25 to $60\ \mu$ in length), radially elongated, and possess thinner walls.

This sclerenchymatous tissue passes rapidly into the parenchymatous tissue of the pulp, which should be next examined. From this it is separated in older fruits by a few layers of cork cells.

Examination of the Pulp

Take a portion of the dry pulp and cut sections from it, which transfer to alcohol. Mount in water, and stain if necessary with a dilute aqueous solution of Bismarck brown so as to

render the thin transparent cell walls more easily visible. The section exhibits very large, thin-walled, more or less rounded, parenchymatous cells, with large intercellular spaces. The areas of contact are flattened and pitted, often showing in consequence a beaded appearance when cut transversely. The



FIG. 131.—Colocynthis Fruit, section of the dry pulp. $\times 65$.

walls are mostly cellulose; here and there a little lignification can be detected.

This tissue is traversed by fibro-vascular bundles containing tubular idioblasts, in which the colocynthin is secreted, but for present purposes these may be neglected.

Examination of the Seed

Preparation and Examination of Sections.—Select some ripe (dark-coloured) seeds, and soak them for several days in water. Split one parallel to the flat surface, and embed one of the halves in cork. Cut transverse sections.

Examine one in glycerin. The epidermis consists of radially elongated cells about $60\ \mu$ long and $20\ \mu$ wide. The outer tangential walls appear to be very thick, but a close inspection shows that the cell walls are covered by a transparent homogeneous membrane. This, according to Hartwich,¹ is the inner epidermis of the pulp of the fruit, the cells of which collapse and become adherent to the seed. The radial walls of the epidermal cells exhibit in section a number (about ten or twelve) of longitudinal bars of thickened wall which alternate with

¹ *Archiv d. Pharmacie* cexx. 584.

as many strips of unthickened wall, the bars tapering from base to apex. Hence in surface view these cells appear conspicuously beaded (fig. 132, *ep*). The contents of the epidermal cells of ripe seeds are brown, and yield the tannin reaction with ferric salts; to them the brown colour of ripe seeds is due. In unripe seeds the epidermal cells are destitute of this brown substance, and in very young seeds they are often collapsed, expanding only when treated with strong solution of potash.

Following immediately upon the epidermis is a mass about $350\ \mu$ thick of sclerenchymatous cells with very thick yellowish striated walls (fig. 132, *scl.*). These cells are small (about $10\ \mu$ in diameter) near the epidermis, but increase in size towards the interior (50 to $70\ \mu$); the walls also increase in thickness until the inner cells are almost completely filled and the cavity can with difficulty be distinguished. These inner cells do not appear well defined, as they are often irregular in shape. The innermost layer is rather sharply distinguished from the others, except in the acute angles of the seed, where all the cells assume an elongated shape with sinuous or jagged walls (see later, isolation by potassium chlorate and nitric acid).

Next to the sclerenchymatous layer is a single layer of reticulated cells about $90\ \mu$ long and $60\ \mu$ wide, but varying considerably (fig. 132,

ret.). They often exhibit dome-shaped projections towards the interior of the seed. The reticulations are lignified. This layer of reticulated cells is followed by a layer of thin-walled



FIG. 132. — Colocynth Fruit, transverse section of part of seed coats. *ep.*, epidermis with outer hyaline layer; *scl.*, sclerenchymatous tissue; *scl.'*, innermost layer of same; *ret.*, reticulated cells; *par.*, parenchyma. $\times 240$.

parenchyma (fig. 132 *par.*), through which runs the raphé, which is easily seen as a fibro-vascular bundle of considerable size near the acute edge of the seed.

Next to the parenchyma is a layer of collapsed cells, in which little structure can be discerned, separated from the embryo by a single layer of distinct flattened cells about 15 to 20 μ long (fig. 133, 4). These latter are probably the inner epidermis of the endosperm, the remainder of which has collapsed to form the tissue referred to. Although but little structure can be discerned in the transverse section, surface preparations will yield useful information concerning this layer.

Proceed next to isolate and examine the tissues of which the seed coats consist.

Separation of the Component Tissues.—Split some seeds longitudinally, remove the kernels, and digest the seed coats in 5 per cent. solution of potash on a water-bath.

Dissect off the epidermis. The alkali swells the outer thickened walls (or collapsed layer of cells) of the palisade epidermis, but the cells can be isolated and the shape well seen.

Dissect or tease off the inner layers; four distinct layers of cells can be distinguished:

- (i) Long, narrow, thin-walled cells, without intercellular spaces (fig. 133, 2).
- (ii) Large, thin-walled, parenchymatous cells with very small intercellular spaces (fig. 133, 3); the walls of the cells appear beaded in optical section and spirally striated in surface view. They often adhere so closely to the preceding layer as to make it appear that the cells of that layer are striated;
- (iii) Rounded cells (fig. 133, 1); these generally adhere to layers 2 and 3, and are with difficulty visible;
- (iv) Delicate, nearly isodiametric cells, each containing a globule of oil (fig. 133, 4). This tissue is probably the epidermis of the remains of the endosperm.

The treatment with potash, while permitting the separation of the softer tissues, is insufficient to separate the lignified cells of the sclerenchymatous layer. To effect this macerate the seed coats with potassium chlorate and nitric acid, wash, and tease out. A most varied assortment of sclerenchymatous cells will

be found. Some are small, regular, and nearly isodiametric; they are derived from the outer portion of the layer; other

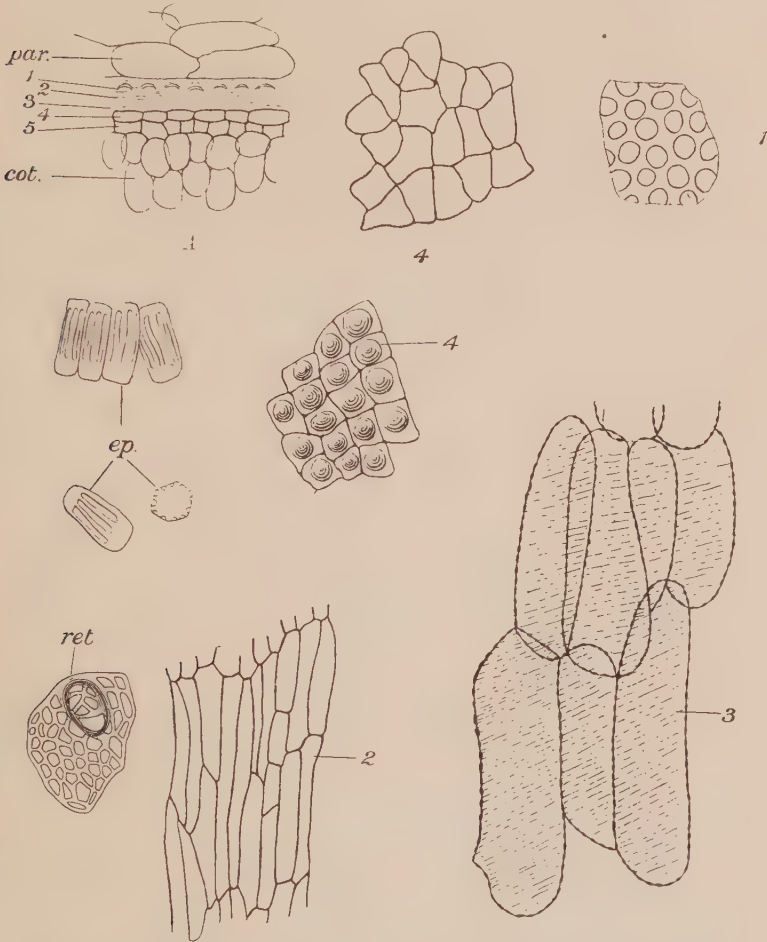


FIG. 133.—Colocynth Fruit, elements of the seed coats. *ep.*, epidermis in section and surface view; *ret.*, reticulated cells within the sclerenchymatous layer, some of the elements of which are shown in figs. 132 and 134; 4, section of margin of cotyledon, with tissues between this and the reticulated cells; *cot.*, cotyledon; *par.*, parenchyma abutting on the reticulated cells; 1, 2, 3, more or less collapsed layers; 4, inner epidermis of remains of endosperm; 5, epidermis of cotyledon. 1, 2, 3, and 4 are also shown in surface view, the last-named with and without oily contents. $\times 240$.

larger and less regular cells are derived from the inner layers, while the innermost row of all consists of very thick-walled

cells with sinuate outline from which forked projections stand out, which dovetail with corresponding indentations in the walls of neighbouring cells. There are also long narrow cells with irregular jagged walls; these are derived from near the

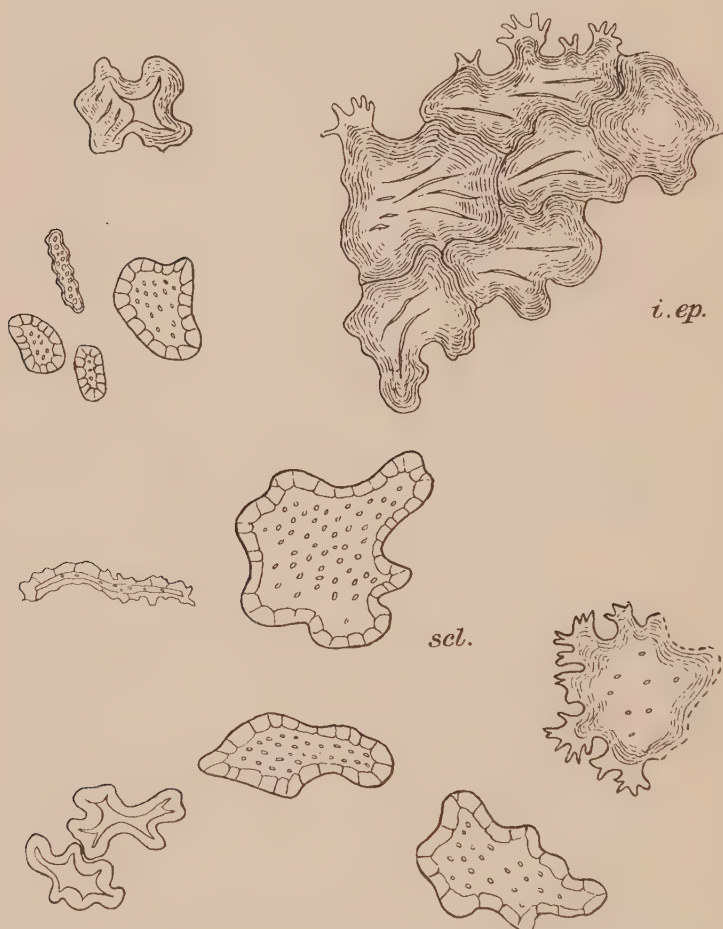


FIG. 134.—Colocynth Fruit, sclerenchymatous cells of seed coat; isolated by potassium chlorate and nitric acid. *i.ep.*, inner epidermis of outer integument. $\times 240$.

micropyle, and from the inner layers near the acute edge of the seed.

Next examine the kernel of the seed.

Open a seed and remove the kernel as carefully as possible.

Embed it in pith, and cut several transverse sections, which should be transferred to a small corked tube containing ether or ether-alcohol. After maceration for fifteen to thirty minutes pour off the ether and transfer the sections with the aid of a little alcohol to a dish.

Mount a thin one in picric acid solution. The cells are filled with very small aleurone grains, each cell containing twenty or more. The grains are ovoid or nearly rounded, and average from 3 to 5 μ in diameter, although they attain as much as 7 μ . Each contains a small rounded body which does not stain with picric acid and resembles a globoid. Irrigate with dilute (0.3 per cent.) solution of potash; the grains dissolve rapidly and entirely, leaving no trace of the globoid body. The cells now exhibit their shape well. On the upper side they are radially elongated and resemble palisade tissue; on the lower they are more nearly isodiametric. They are covered by an epidermis composed of small delicate thin-walled cells.

Mount a section (not previously defatted) in chloral hydrate; oil globules exude in abundance.

Mount a defatted section in iodine water or dilute iodopotassium iodide; there is no starch present, at least in ripe seeds.

No calcium oxalate can be found, either in the seed coats or in the kernel.

Most of the seeds that are present in the commercial drug are unripe; the student should therefore also cut sections of unripe seeds, and compare them with the ripe. The following differences may be observed.

The epidermis of the seed coat is less developed; the bar-like thickenings have often not formed; sometimes even the epidermal cells are indistinct, and the sclerenchymatous cells are much less thickened. A study of the seed coats of unripe seeds contributes materially to the proper understanding of that of the ripe seeds.

Diagnostic Characters.—The following cells and cell contents are the most important from a diagnostic point of view:

1. The large thin-walled cells of the pulp; powdered colocynth pulp should consist principally of fragments of these; they stain blue with chlorzinciodine.

2. The epidermis of the pericarp; this, if present to any

appreciable extent in powdered colocynth, would indicate the use of unpeeled or badly peeled fruits ;

3. The palisade epidermis of the seed coat, with its characteristic thickenings ; the fact that these thickenings do not branch towards the apex of the cell distinguishes this seed from certain other cucurbitaceous seeds ;

4. The sclerenchyma of the seed coat, especially the cells of the innermost layer ;

5. The spirally striated cells ; most cucurbitaceous seeds contain these.

Additional means of determining the presence of seed in the powdered drug may be found in

6. The aleurone grains ;

7. The presence of oil.

Neither pericarp nor seed contains either starch or calcium oxalate.

Examination of the Powder

Proceed next to the examination of the powdered fruit, taking care to obtain powdered *fruit* and *not pulp*.

Moisten a little with alcohol, add water, and examine. Observe in this preparation abundant *débris* of parenchymatous tissue evidently consisting of very large cells. Sometimes these fragments exhibit pitted areas or transverse walls, but such are not very readily seen. There is also a quantity of granular matter present and yellowish masses which do not readily show their structure ; they are fragments of sclerenchymatous tissue, but they are better examined in another medium. Allow chlorzinciodine to flow on ; the colourless fragments of parenchyma turn bluish violet.

Moisten another portion with alcohol, allow this to become nearly dry, and add a very small drop of picric acid solution ; mix well, and after a minute or two add a drop of glycerin ; mix, and examine. The small oval aleurone grains can be easily found ; they are present in numbers, and are readily detected by their yellow colour. The small rounded globoid (?) appears faintly orange red in colour. Carefully examine this slide for fragments of the cotyledons. These may be detected by the now yellow aleurone grains with which the cells are packed ; they are not, however, very readily observed.

Warm another portion in chloral hydrate. The sclerenchymatous tissue is now very conspicuous ; it generally has a yellow colour. It is present as isolated cells, or groups of cells, or

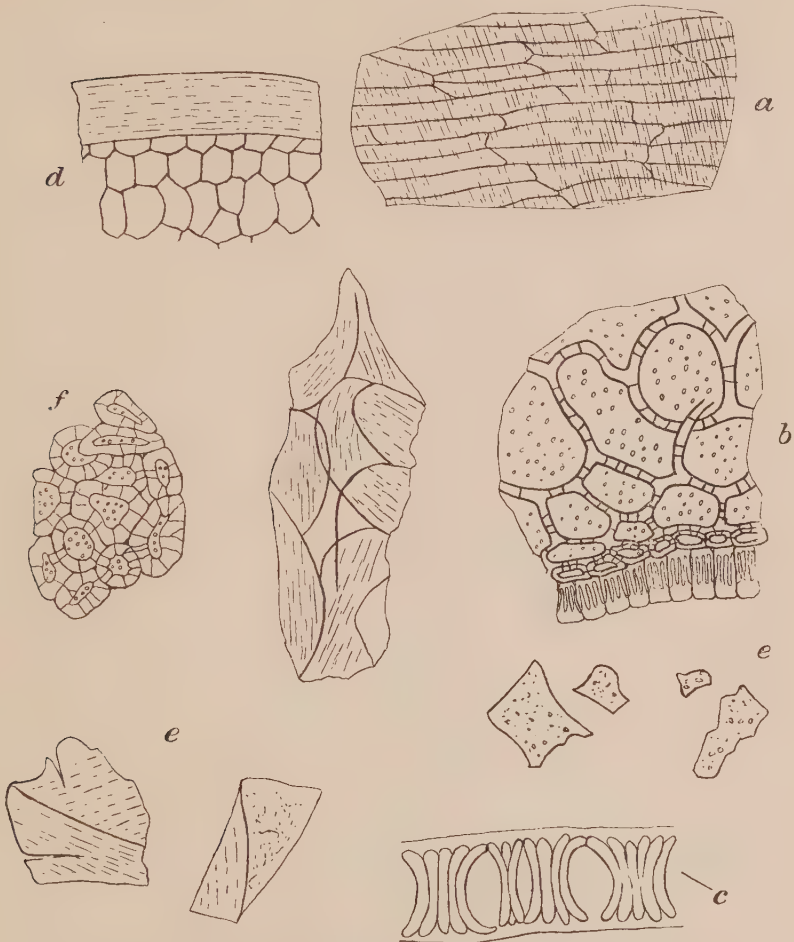


FIG. 135.—From Powdered Colocynth Fruit. *a*, striated cells of inner seed coat (compare 2 and 3, fig. 133); *b*, epidermis and subjacent sclerenchyma of unripe seed; *c*, inner epidermis of outer integument of unripe seed; *d*, fragment of cotyledon with inner layers of seed coat; *e*, fragments of cells of pulp; *f*, sclerenchyma of nearly ripe seed. $\times 240$.

transverse fragments of the seed coats from the epidermis to the innermost sclerenchymatous layer. Observe such cells or fragments of sclerenchymatous tissue as have much thinner

walls and especially differ in the innermost layer; they are derived from unripe seed, and are commonly more numerous than those derived from ripe seed. These fragments are not very readily distinguished from the sclerenchyma of the rind, but the cells of the latter usually exhibit distinct radial elongation and are more angular and comparatively thin-walled. Some of the sclerenchymatous cells are long and narrow, with jagged edges; these come from the acute edge of the seed.

The delicate striated cells from the collapsed layers are very readily found: they generally occur in fragments of considerable size exhibiting their surfaces (fig. 135, *a*).

The epidermis of the seed coat may also be found; the cells are often in profile, but sometimes in surface view; although the outer wall is more or less swollen, the bar-thickenings are always conspicuous and are very characteristic.

The epidermis of the fruit may sometimes be detected; it is specially easily recognised when exhibiting its transverse section, but should be present only in very small quantity.

Stain another portion with phloroglucin and hydrochloric acid; the sclerenchymatous cells are coloured deep red.

Examine next the powdered pulp of commerce. Here too fragments of the tissue of the seed may be found, but they should not be so numerous as to raise suspicion of adulteration.

Mount a little iodine water or chloral iodine to prove the absence of any but occasional very minute isolated starch grains (derived from very young seeds).

Capsicum Fruit (Chillies)

Source.—The fruit of *Capsicum minimum*, Roxb.

Preparation.—Capsicum fruit should be divided, for complete examination, into the following parts, and each examined separately:

- | | |
|---------------------------|---------------------|
| (<i>a</i>) Pericarp; | (<i>d</i>) Calyx; |
| (<i>b</i>) Dissepiment; | (<i>e</i>) Stalk. |
| (<i>c</i>) Seed; | |

Examination

(*a*) **Pericarp.**—Select a few good Zanzibar or Sierra Leone chillies, and soften them by exposing them to a moist atmosphere for twelve hours. Carefully separate the pericarp, cut

into suitable strips, embed in pith, and cut transverse sections. Examine in chloral hydrate.

Observe the outer epidermis. The outer tangential walls are much thickened, and so also are the radial walls, but only for about two thirds of their length, the remaining inner third

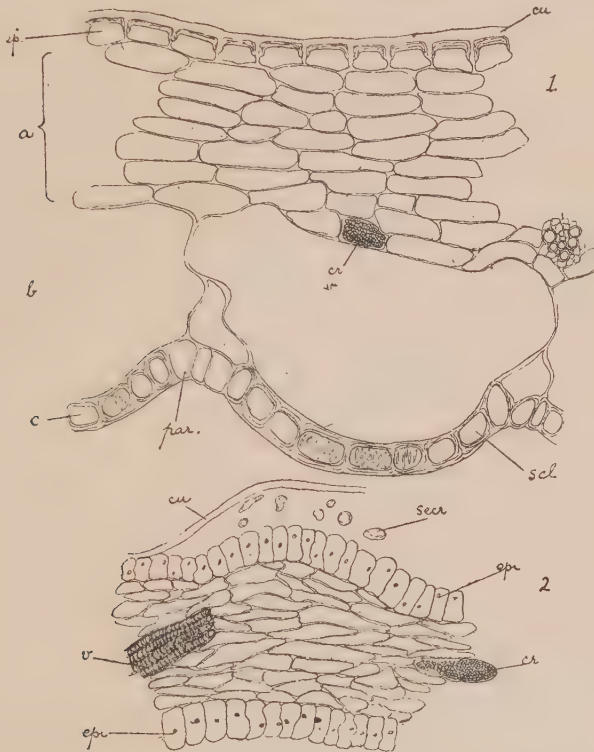


FIG. 136.—Capsicum Fruit. 1, transverse section of pericarp; *a*, parenchyma; *b*, large-celled layer of same; *c*, inner epidermis; *cr.*, sandy crystals of calcium oxalate; *par.*, parenchymatous cells, and *scl*, sclerenchymatous cells of inner epidermis. 2, transverse section of dissepiment; *cr.*, sandy crystals of calcium oxalate; *cu*, cuticle, raised by the secretion, *secc*; *epi*, epidermis; *v*, vascular bundle. $\times 170$. (Wallis.)

being thin. Below the epidermis is parenchymatous tissue containing much reddish-coloured oil, and here and there a small bicollateral bundle and cell filled with sandy calcium oxalate. Between this parenchyma and the inner epidermis there is a row of very large cells, so large that they might be mistaken for spaces. The inner epidermis itself is composed

of two kinds of cells—viz. sclerenchymatous cells, with thick, pitted, lignified walls, and thin-walled parenchymatous cells. The latter occur over the radial walls of the large subepidermal cells, and hence in surface sections exhibit a reticulate distribution corresponding to the walls of the cells over which they are situated; the sclerenchymatous cells occupy the spaces between, and the groups of these cells correspond, therefore, to the cavities of the large cells.

From another fruit, softened as described, separate the pericarp; observe with a lens the bladdery appearance of the inner surface, due to the large cells just alluded to. With dissecting needles and forceps strip the epidermis from the inner surface and examine in chloral hydrate, taking care that the cuticle is uppermost. If observation is impeded by the oil, which is often present in considerable quantity, remove this by maceration in ether-alcohol. The sclerenchymatous cells can now easily be seen in surface view; they are approximately isodiametric, have thickened, pitted, lignified, sinuous walls, and are arranged in elongated oval groups between which there are narrow strands of delicate parenchymatous cells. The latter are not very easily made out, and require careful adjustment of the light and focus.

Warm a fragment of the pericarp in chloral hydrate; the upper epidermis shows well; the cells are mostly four-sided, and have a delicately striated cuticle; they often fall into rows of seven or more, which appear to have been formed by the transverse division of one large elongated cell—a peculiarity that is characteristic of this variety of *Capsicum*.

(b) **Dissepiment.**—Cut open another fruit, and carefully remove the thin membranous dissepiment. Examine the surface in chloral hydrate after warming. The epidermis is formed of thin-walled polygonal cells, from which the cuticle is in great part separated, forming an indistinct, structureless, crumpled membrane over the epidermal cells. Among the parenchymatous tissue cells filled with sandy crystals are to be seen.

A transverse section through the delicate dissepiment shows that the cuticle has been raised from the epidermis in places by the secretion from the epidermal cells of oily drops; these are said to contain the capsaicin to which the fruit owes its pungency.

(c) **Seeds.**—Soak a few seeds in water until sufficiently soft to cut. Embed one in pith, and cut transverse sections with a sharp razor; if it is not held sufficiently firmly by the pith, fix it between the two halves of a velvety cork.

Examine the sections in chloral hydrate. Observe the epidermis carefully. It is composed of cells that exhibit a very

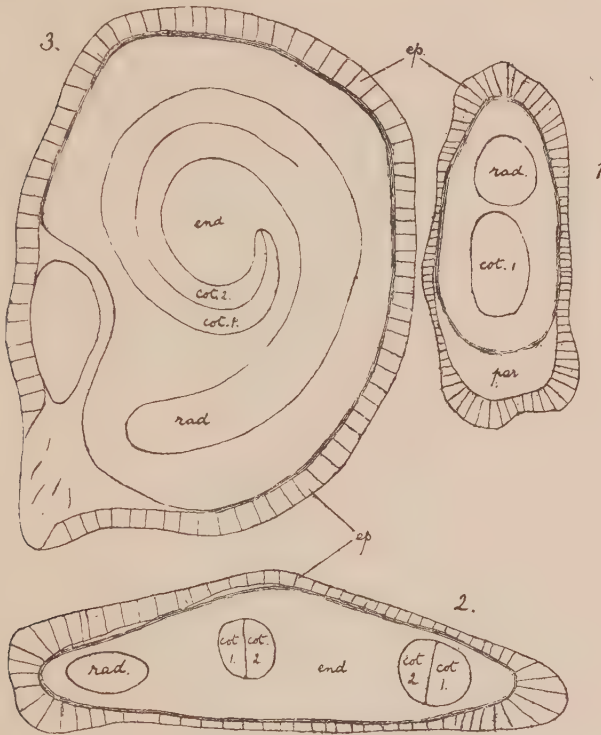


FIG. 137.—Capsicum Fruit, sections of the seed. 1, transverse; 2, longitudinal cut at right angles to the flat surfaces; 3, longitudinal, parallel to the flat surfaces. (Wallis.)

remarkable thickening. The inner tangential wall is moderately thickened in the centre, more strongly in the angles, but on the radial walls the thickening gradually diminishes until, near the outer tangential wall, it is very slight. The section therefore exhibits an irregular horseshoe thickening.

Stain a section with chlorzinciodine; on the outside there is a delicate cuticle; next to this a layer of cellulose, and

within the layer of cellulose a lignified layer; the general cavity of the cell is also lined with a layer of cellulose.

The cells on the edge of the seed are larger and more strongly radially elongated than those on the flat sides.

Fix a seed by its flat surface on a cork, using a little mucilage; allow it to dry; cut surface sections, and examine

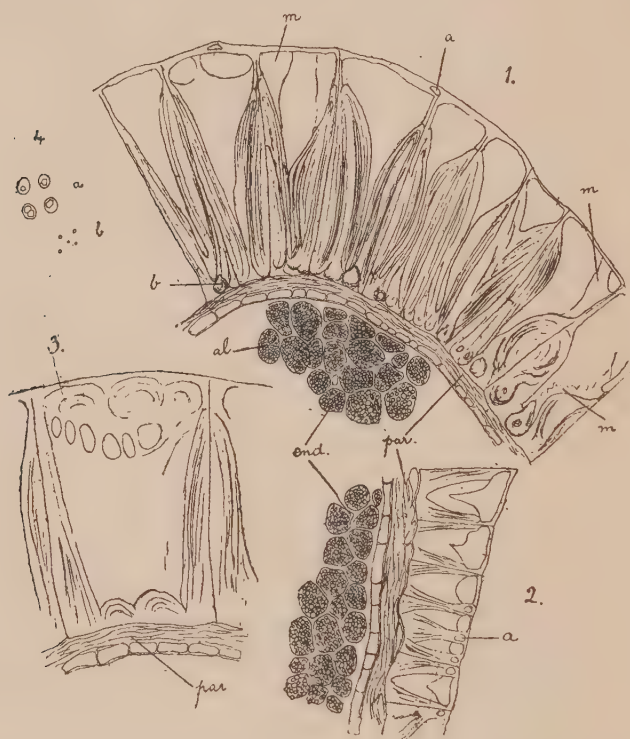


FIG. 138.—Capsicum Fruit. 1, transverse section of seed coat and portion of endosperm, at edge of seed. 2, the same from flat surface. 3, seed coat at edge of seed, cut parallel to the flat surface; *a*, processes from the upper surfaces of epidermal cells; *b*, processes from the lower surfaces of the same; *al.*, aleurone grains; *end.*, endosperm; *m*, delicate cellulose membrane in epidermal cells; *par.*, collapsed parenchyma of seed coat. All $\times 150$. 4, aleurone grains, more highly magnified; *a*, before, *b*, after treatment with potash. (Wallis.)

in chloral hydrate. The cells appear thickened, and the outline wavy.

Split a few seeds parallel to the flat surface and also transversely; remove the endosperm; macerate the fragments with potassium chlorate and nitric acid; wash; tease out, and

examine the separated cells. They have the same appearance as they showed in surface section, but they exhibit, in addition, little irregular projections which dovetail with those of neighbouring cells.

Examine the kernel as directed for colocynth seeds; it consists of thin-walled cells, in which aleurone grains and oil are the principal reserve materials.

Calyx and Stalk.—Examine these by the methods previously detailed for leaves and stems.

The upper epidermis of the calyx is characterised by numerous multicellular glandular hairs containing a yellowish secretion; stomata occur on the under surface only, and each is surrounded by three or four cells, of which one is smaller than the others. The mesophyll contains cells filled with sandy crystals of calcium oxalate.

The stalk has an epidermis consisting of elongated cells and bearing an occasional glandular hair; the pericycle contains well-developed fibres.

Powder

Powdered chillies may be examined as follows:

(1) In water or dilute glycerin. Note the abundance of red globules of oil. Fragments of the epidermis of the seed and pericarp may be observed, but are better examined in the following preparation. Aleurone grains are not easy to identify.

(2) Warm a little in chloral hydrate, cool, and examine. In this preparation fragments of the epidermis of the seed coat with its remarkable sclerenchymatous cell walls of yellowish colour are usually very conspicuous. Sometimes they exhibit their surface, especially if the fragments are large, but smaller ones or isolated cells often present their section. The inner epidermis of the pericarp, with its thick-walled lignified cells, is also easily found. Sometimes these cells are attached to the non-lignified parenchyma, with which they alternate, but more often they are separated. The striated outer epidermis is also to be found, but not quite so easily. Portions of the endosperm, the cells of which have rather thick walls, are not difficult to detect, while fibres and vessels from the stalk, calyx, &c., are scattered in every preparation.

(3) Defat a little of the powder with ether-alcohol, dry, and

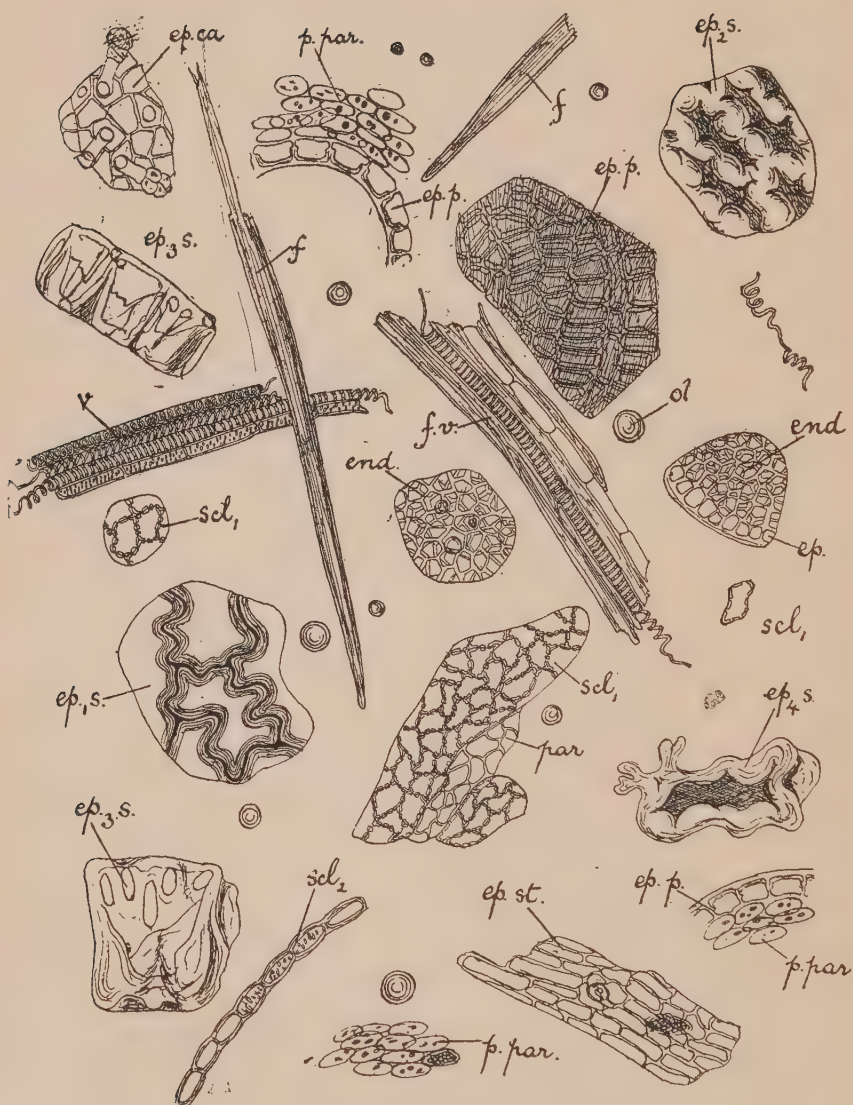


FIG. 139.—*Capsicum* Fruit. Powder. *end.*, endosperm; *ep.*, epidermis of same; *ep.ca.*, upper epidermis of calyx; *ep.p.*, outer epidermis of pericarp; *ep₁s.*, epidermis from flat surface of seed; *ep₂s.*, epidermis from edge of seed; *ep₃s.*, epidermis of seed, side view; *ep₄s.*, isolated epidermal cell of seed coat; *f.*, sclerenchymatous fibres; *f.v.*, fibro-vascular bundle; *ep.st.*, epidermis of stalk; *o.*, oil; *par.*, parenchyma of inner epidermis of pericarp; *p.par.*, parenchyma of pericarp; *scl₁*, sclerenchyma of inner epidermis of pericarp, seen from above; *scl₂*, the same, side view. $\times 108$. (Wallis.)

mount in chloral hydrate. Compare this preparation with No 2.

(4) Decolourise a little of the defatted powder, wash, and treat as follows :

- (a) With Soudan red : the portions of epidermis bearing a cuticle stain red ;
- (b) With phloroglucin and hydrochloric acid : lignified cell walls are stained.

Fruits of other Species of Capsicum.—Wallis¹ has also examined the fruits of *C. annuum* as well as those known in commerce as Japanese chillies, the botanical origin of which has not been definitely determined. The following table shows the chief features by which these fruits may be distinguished from one another either in the entire or powdered state.

	C. MINIMUM	C. ANNUM	JAPANESE CHILLIES
Epidermis	Thick and straight-walled rectangular cells with few pits; often arranged in groups of five to seven in a row and with a uniformly striated cuticle. Size of cells, 25μ to 60μ in either direction.	Irregular polygonal cells with evenly thickened walls, traversed by numerous well-marked simple pits. The cuticle shows striated ridges. Size of cells, 60μ to 100μ long, and 25μ to 50μ wide	Cells with strongly thickened walls and a radiate lumen. The pits only rarely penetrate the whole thickness of the wall. No visible striation. Size of cells, 30μ to 80μ long and 15μ to 45μ wide
Hypodermis	Delicate cells with thin cellulose walls	Several layers of cuticularised collenchymatous cells, having a rounded outline and very few pits	A single layer of regular polygonal cells with cuticularised fairly thick walls, traversed by numerous pits, which give them a beaded appearance

Black Pepper

Source.—The unripe fruit of *Piper nigrum*, Linn.

Preparation and Examination of Sections.—Soak a number of peppercorns in water for about twelve hours, when they will be sufficiently softened for examination.

Examine with a lens, and find the scar that indicates the point of attachment to the stem; at the apex of the fruit the

¹ *Pharmaceutical Journal*, vol. 69, p. 3.

remains of the stigmas can generally be discerned. Cut the fruit transversely to this axis, and then each half again at right angles to the transverse surface. Embed one of these quarters in



FIG. 140.—Black Pepper. *A*, transverse section; *B*, vertical section, showing pericarp, *sch*; perisperm, *p*; endosperm, *en*; and embryo, *e*. Magnified. (Tschirch.)

pith, so that the half of the transverse section is presented for cutting. When cutting the sections take care that the edge of the razor cuts the pericarp first (this should therefore be towards the operator's left and the perisperm towards his right).

Examine a section in water under the low power; the brownish tissue of the pericarp

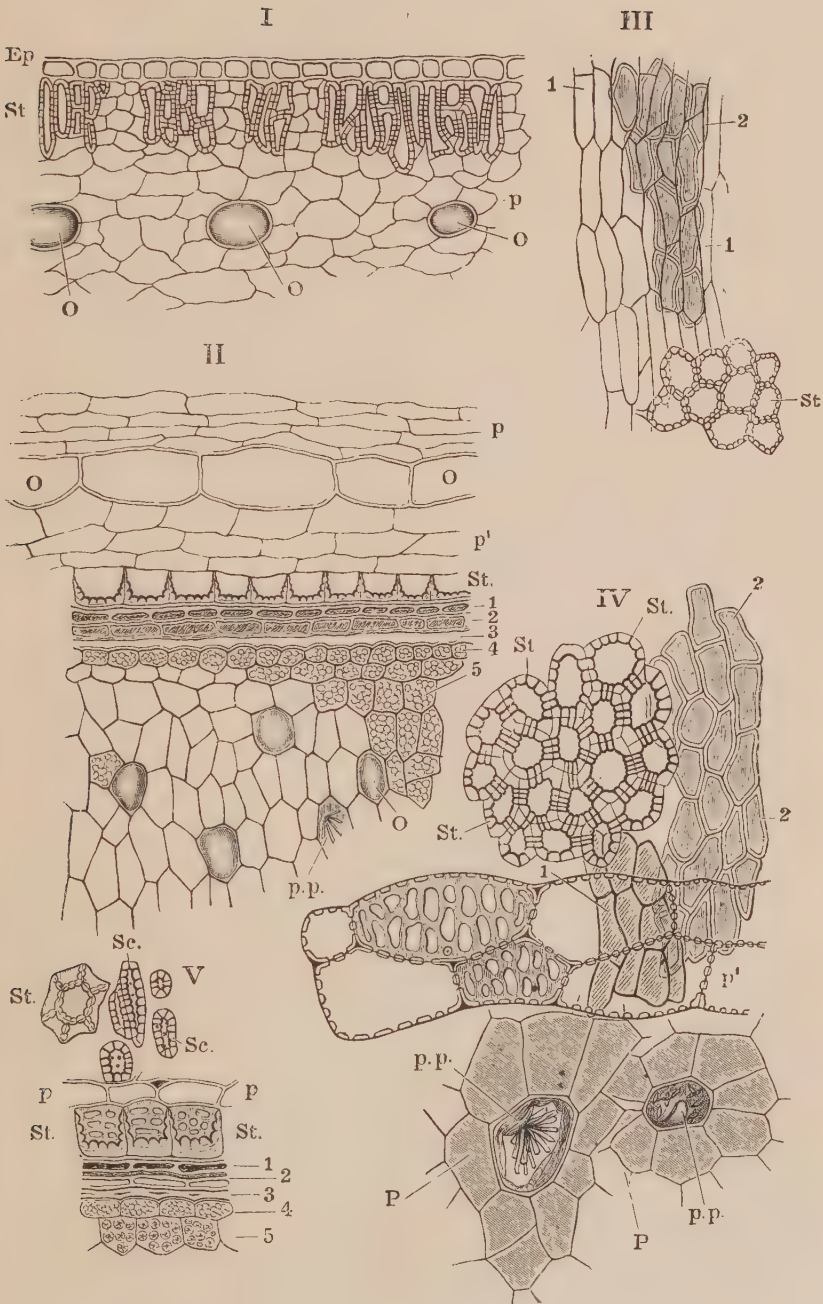
is well differentiated from the whitish perisperm, the cells of which are polygonal and packed with minute starch grains.

Mount a thin section in solution of potash, and warm gently till the starch is gelatinised; examine first under the low, then under the high power.

The epidermis is formed of small cells covered with a thick cuticle; they are often best seen near the ends of the section where it is thinnest; they contain a brownish granular substance in which minute prismatic crystals of calcium oxalate are embedded; six or eight such crystals can often be seen in each cell (especially in surface preparations).

Below the epidermis, sometimes immediately abutting upon it, sometimes separated from it by a row of small parenchymatous cells, is a layer of sclerenchymatous cells. This layer is not continuous, but interrupted at intervals by small groups of parenchyma. The sclerenchymatous cells themselves vary

FIG. 141.—Black Pepper. I., transverse section of the outer portion of the pericarp; Ep, epidermis; St, sclerenchymatous cells of the hypodermal (or sub-hypodermal) layer; p, parenchymatous cells; O, oil cells. II., transverse section of the inner portion of the pericarp and part of the perisperm of the seed; p, parenchyma; O, oil cells; p', subjacent parenchyma; St., inner layer of sclerenchymatous cells; 1, 2, 3, layers of the seed coats; 4, epidermis of perisperm; 5, perisperm with oil cells, O, in one of which crystals of piperine can be seen. III., sclerenchymatous cells of the inner layer, together with the subjacent seed coats; 1 and 2, surface view. IV., surface view of some of the tissues; St., sclerenchymatous cells of inner layer; 1 and 2, subjacent seed coats; p', the reticulated parenchymatous layer exterior to St; P, parenchyma of the perisperm, with starch and oil cells, the latter containing crystals of piperine. V., transverse section of the inner layers of the pericarp, lettering as in II. (Vogl.)



considerably, both in size and in shape. Some are isodiametric, 15 to 20 μ in diameter, and square or rounded in outline; but the majority are radially elongated, and often attain 100 μ in length and 20 μ in width. They often exhibit brown contents. These cells are characteristic of pepper, and should therefore be carefully examined. The parenchymatous tissue of this region often contains brown granular matter in which numerous small crystals of calcium oxalate can be detected. The latter are especially easily visible when a section cleared by potash or chloral hydrate is examined with polarised light under the high power.

Following upon this outer sclerenchymatous zone is a parenchymatous tissue which constitutes the bulk of the pericarp and is rather sharply differentiated into an outer and inner portion separated by a dark, often greyish or brownish line, which on careful examination is seen to consist simply of compressed thin-walled cells. This region of the pericarp is traversed by fibro-vascular bundles, which in transverse section exhibit a few small spiral vessels and often sclerenchymatous fibres, the nature of which can be determined later from a potash maceration preparation.

The outer portion of this parenchyma is composed of about twelve rows of tangentially elongated parenchymatous cells, which contain, as may be determined by examining a section in iodine water, numerous minute scattered starch grains. Here and there in this tissue an oil cell may be found containing a globule of oil; these are most evident in moderately thick sections after treatment with potash.

The inner portion of the parenchyma is especially characterised by the presence of a large number of oil cells, which are arranged in an almost continuous ring. The innermost row or two of the cells of this tissue are often reticulately thickened (especially in riper fruits); they abut directly upon a single row of cells that are conspicuous by reason of their bright colourless walls. These conspicuous cells exhibit a very remarkable horseshoe thickening on the radial and inner tangential walls, coarse pits being visible near the points of the horseshoe. This layer is the inner epidermis of the pericarp, and is of great importance, as it is one of the most characteristic layers of the pepper fruit.

Between the horseshoe cells and the perisperm is a narrow brown ring, which, in favourable sections and under a high

power, can be resolved into three distinct layers—viz. an outer pale brown, a middle dark brown, and an inner colourless one. As a rule, these layers do not exhibit more than indications of cellular structure, but such indications become more distinct after treatment with Schulze's maceration mixture, &c.; they are, however, best examined in surface sections or potash preparations. These layers constitute all that remains of the seed coats.

Examine a section in water. The perisperm is principally composed of large thin-walled polygonal cells packed full of minute starch grains. Among these cells numerous others may be distinguished by their yellowish oily contents. These are oil cells. They are characterised by the blood-red colour they assume with concentrated sulphuric acid, a reaction due to the piperine they contain, which may occasionally be found in prismatic crystals embedded in the oily contents of the cells.

The perisperm cells that abut on the seed coats are smaller and contain aleurone grains.

The starch occurs in minute simple grains or larger rounded or ovoid compound grains, the latter consisting of a large number of minute component grains. To examine the starch, slightly crush a section and observe the minute grains. Allow chloral hydrate to flow on to a very thin section; large oval or rounded compound grains can then easily be seen embedded in a mass of simple grains. After the starch has been gelatinised many of the cells will be seen to contain a prismatic crystal of calcium oxalate as well as a small body of irregular form and unknown nature.

Examination of Surface Section.—Select a smooth (nearly ripe) fruit, embed it in pith so that the surface of the fruit is just below that of the pith, and cut a series of surface sections. Transfer them all to a slide, keeping the upper surface uppermost, clear, and examine.

The first section should exhibit the epidermis in surface view; the cells, which are often best seen near the edge of the section, are small, rounded-polygonal in shape, and contain numbers of minute crystals of calcium oxalate.

The second section should exhibit the distribution of the outer sclerenchymatous cells; they occur in patches separated from one another by brownish parenchymatous cells, which, like the epidermal cells, contain calcium oxalate.

The following sections should show, near the middle, the outer parenchymatous tissue, the inner parenchymatous tissue, the horseshoe layer, the seed coats, and the perisperm successively.

The horseshoe layer should be particularly examined, as it always presents its surface view in powdered pepper. The appearance of the cells varies a little with the point at which they are focussed; with high focus they appear rather thin-walled and pitted; as the focus is lowered the walls appear thicker and the pits disappear.

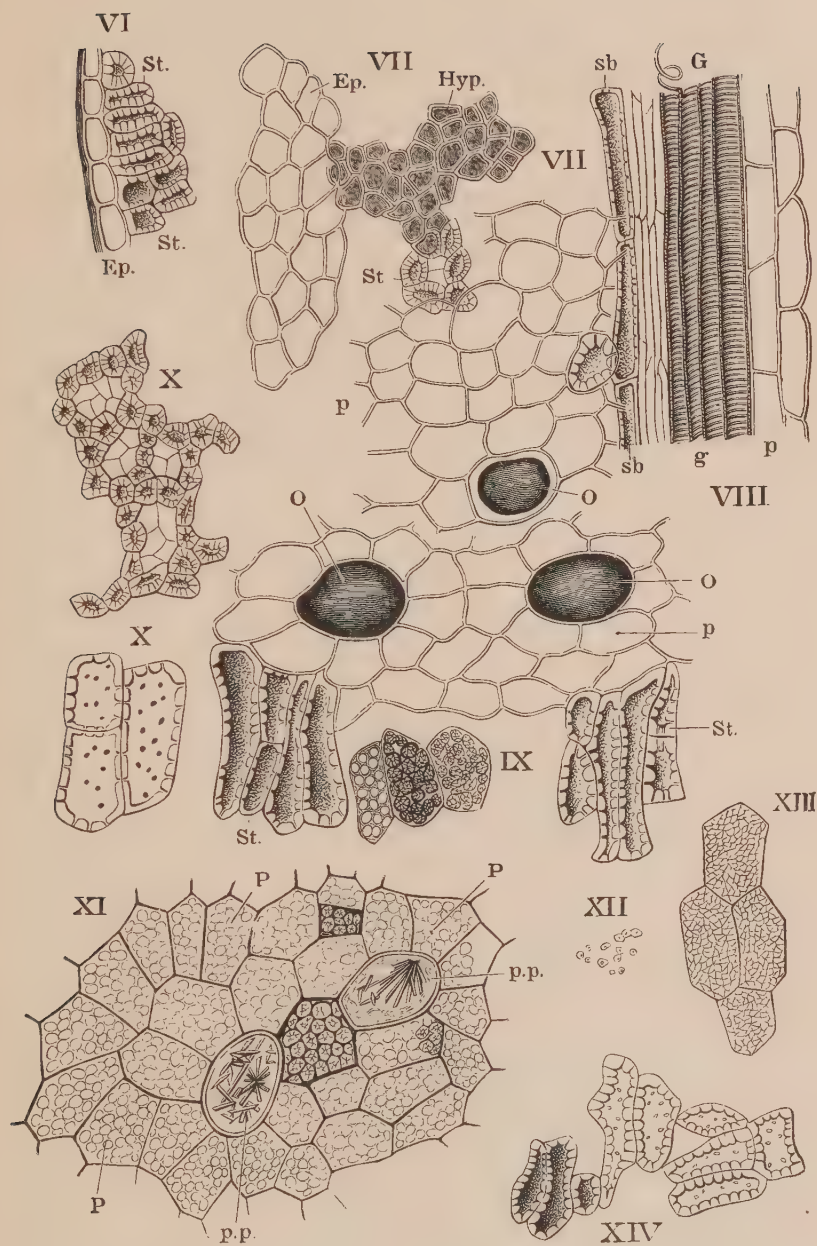
Of the layers of the seed coats the middle dark layer is the most important; it may be recognised by its dark reddish-brown colour, the cells being elongated polygonal in shape. These layers of the seed coat may be further examined in a potash maceration preparation.

Examination by Maceration in Potash.—Digest a few peppercorns in solution of potash on a water-bath; strip the pericarp and seed coats from one of them, and disintegrate as thoroughly as possible with the needles. Examine, and identify the various tissues and elements by comparison with the sections.

Examination of Powdered Pepper.

- (1) Mount a little in water or dilute glycerin. The bulk of the powder consists of angular colourless fragments of perisperm, which present a granular appearance, due to the minute starch grains with which the cells are packed. In addition to these fragments there are to be found the cell contents of some of the cells, which have fallen out intact, and also numerous starch grains, which are either isolated or in groups of a few; these grains can be identified as pepper starch.

FIG. 142.—Black Pepper (*continued*). VI, epidermis, Ep., with subjacent sclerenchymatous cells, St. VII, Ep., epidermis; Hyp., hypoderma; St., hypodermal sclerenchyma, all in surface view; p, parenchyma of pericarp, with oil cells, O; and hypodermal sclerenchymatous cells, St. VIII, fibro-vascular bundle in radial section; g, vessels; p, parenchyma; sb, sclerenchymatous cells. IX, perisperm cells with starch, on the left a cell in which the starch has been gelatinised. X, upper figure, hypodermal sclerenchyma, surface view; lower figure, sclerenchymatous cells from the mesocarp. XI, portion of the perisperm. XII, isolated starch grains. XIII, cells of the perisperm, with starch. XIV, sclerenchymatous cells. (Vogl.)



Irrigate with iodine water in order to make sure that these grains really are starch.

In this preparation fragments of the reddish-brown seed coats and other *débris* can be seen, but they are better examined after removal of the starch.

- (2) Moisten a little with a small drop of alcohol, allow it to stand a few moments, and add a small drop of dilute glycerin. The preparation will become cloudy, owing to the separation of volatile oil. Cover, and after five minutes examine again. Abundance of long narrow prismatic crystals will be found, either isolated or arranged in radiating groups; these are crystals of piperine.
- (3) Boil 1 gramme for ten minutes with 20 c.c. of water to which 2 c.c. of hydrochloric acid (1:16) have been added; allow the cell *débris* to deposit, wash once with dilute (about 1 per cent.) solution of potash, and finally with water. Mount a little in chloral hydrate, and examine. Observe the following characteristic portions of the pepper fruit.
 - (a) *Fragments of the parenchymatous tissue of the perisperm.* The cells are large, elongated, and often angular, and have very thin walls without visible pits. Here and there they appear to possess dull granular contents (remains of the starch), and occasionally a rounded cell with refractive walls may be found (oil cell), in which sometimes a globule of oil may be seen.
 - (b) *Similar fragments from the pericarp;* in these the parenchymatous cells are smaller, the oil cells are also smaller; the fragments often have a pale brownish colour, and contain the small fibro-vascular bundles.
 - (c) *Fragments of the seed coats.* These are more conspicuous in white pepper, which is prepared from riper fruits than black pepper. They have a bright reddish-brown colour, and are easily seen. Discernible in these fragments are usually:
 - a.* The hyaline layer, colourless, with very distinct cell walls.
 - β.* The inner seed coat, dark reddish-brown, cells often less distinct.

- γ. The outer seed coat, yellowish brown, visible here and there.
- (d) *Fragments of the inner sclerenchymatous layer.* They are usually brownish in colour from the seed coats which adhere to their lower (inner) surface; they often exhibit, especially near the edges of the fragments, the large reticulated parenchymatous cells that abut upon their upper (outer) surface. The small polygonal cells of which this sclerenchymatous layer consists and their pitted walls render it easy to identify.
- (e) *Fragments of the outer sclerenchymatous layer.* These are absent or very rare in white pepper, but are numerous in black. They are conspicuous by reason of their dull, dark, brownish or yellowish colour (not bright reddish-brown). They usually exhibit their surface view, and are then sharply characterised by the small sclerenchymatous cells which present somewhat varying, often irregularly polygonal, sections, and are arranged in small groups separated by dark-coloured parenchyma. The epidermis often adheres to them, but is not readily seen.
- (f) *Isolated sclerenchymatous cells*, or small groups of such. These are derived chiefly from the outer sclerenchymatous layer. Long narrow ones may occasionally be found; these are the sclerenchymatous cells that accompany the bundles in the pericarp.

Wheat

Source.—The fruits of *Triticum sativum*, Lam., and other species of *Triticum*.

Preparation.—Examine a grain of wheat with a lens; it is ovoid in shape. One side is rounded, while on the other there is a deep furrow. Near the apex of the fruit there is a tuft of hairs.

Cut a grain longitudinally and examine the section with the lens; near the base the embryo can be seen.

Transverse Sections.—Soak some grains in water until sufficiently soft to cut (twelve hours or more; they may be preserved after softening in a mixture of equal parts of 70 per

cent. alcohol and glycerin); embed one in pith, and cut a number of transverse sections, taking care that the pericarp of the grain is cut as thinly as possible. Transfer these sections to alcohol. Examine one in dilute glycerin: the abundant starchy endosperm is surrounded by several narrow layers of cells varying from yellow to brown in colour; these layers comprise the tissues of the pericarp and the closely adherent seed coats.

Mount one in chloral hydrate, warming gently to clear it. Examine carefully under the high power, commencing with the outermost layer (epidermis of the pericarp) and passing to a narrow dark brown line (seed coats), which is usually very distinct, and finally to the large thin-walled cells of the endosperm.

The three or sometimes four outermost layers of cells are often more or less collapsed to form a pale yellow tissue in which indications only of cell cavities can be detected. If this is the case, they must be expanded by warming in chloral hydrate or in potash, but it must be remembered that the use of the latter reagent may cause considerable swelling of the walls.

The epidermal cells are oblong, and possess thickened tangential but thin radial walls; (fig. 143, 1 and 10, 1); the next following two rows of cells, constituting the hypoderma, are similar in appearance, and both these and the epidermal cells have lignified walls. Pits are difficult to see in the transverse section. Next to this tissue is a row of parenchymatous cells which are larger and have thin walls. These cells are also more or less collapsed, and appear not to be present in every part of the section; they are often difficult to see in transverse sections (fig. 143, 10, 4).

Following upon this layer is a single row of (in transverse section) elongated cells that exhibit conspicuous pitting. They average about 110 to 130 μ in length and 12 to 15 μ in width; the walls are yellowish in colour and lignified (fig. 143, 10, 3).

Between this and the seed coats are tubular cells which appear as rounded or flattened rings in transverse section. These cells are not regularly arranged, but distributed in an irregular manner, or at least are only to be seen at irregular distances, and best in sections that have been expanded by warming in potash. They are the remains of the inner epi-

dermis of the pericarp, the constituent cells of which are tubular in shape and have become separated from one another during the development of the fruit; hence their irregular distribution (fig. 143, 10, 6).

The narrow brown line that represents the collapsed seed coats is often apparently homogeneous, but under the influence of caustic potash indications of radial walls can often be detected (fig. 143, 10, 7).

Abutting upon the seed coats are the remains of the nucellus, which appear as a narrow hyaline layer. After warming in chloral hydrate the tangential walls of the constituent cells swell and exhibit distinct striations; the radial walls also become visible. Two rows of such cells can be seen (fig. 143, 10, 8).

There follows next the aleurone layer, a single row of cells which constitutes the outermost layer of the endosperm (fig. 143, 10, 9). The cells of which it consists are large (45 to $60\ \mu$ by 30 to $50\ \mu$) and radially elongated. The walls are rather thick. The cells are packed with small rounded aleurone grains (picric acid reaction on a fresh section), associated with oil-containing plasma (sulphuric acid reaction). The cells of the endosperm are large and have very thin walls; they are filled with starch grains (see p. 14) associated with minute aleurone grains (fig. 143, 10, 10).

Examine the furrow that runs down one side of the grain, and in the sections will of course be cut transversely. The seed coats can easily be traced by reason of their brown colour; they follow the course of a **W**. The space of the central triangle is occupied by thin-walled parenchymatous tissue, apparently an expansion of the tissue of the nucellus. Here the aleurone layer has often broken away from the nucellus. At the apex of the triangle there is a group of thin-walled cells filled with a brown amorphous substance; these cells are part of the seed coat.

The two lateral triangles of the **W** are occupied by collapsed cells derived from the outer layers of the pericarp; the sclerenchymatous cells can be traced nearly to the apices of these triangles; they resemble the corresponding cells from other parts of the grain.

Radial Sections.—Cut next longitudinal sections. The pericarp exhibits a structure similar to that observed on the

transverse section, but the epidermal and hypodermal cells appear much longer, while the sclerenchymatous cells are small and exhibit an almost isodiametric section; the cells of the seed coats are often a little more distinct.

At the base, on the curved side of the fruit, is the embryo. This is built up of small thin-walled cells filled with plasma and oil.

Near the apex of the grain there are numerous long, one-celled, thick-walled, tapering hairs which must be closely examined, but are better seen in a surface preparation.

Surface Sections.—Next cut surface sections from several fruits; immerse them in spirit and then examine in dilute glycerin or chloral hydrate, taking care that the epidermis is uppermost. Focus down through one layer to the next, as may be necessary.

The first layer is the *epidermis*; it consists of elongated cells (often 100 to 200 μ long and 25 to 50 μ wide), with rather thickened pitted longitudinal walls, but thin transverse walls, the latter being often more or less oblique, so that the cells are somewhat pointed (fig. 143, 1).

The second layer consists of similar cells, but the transverse walls are often more distinctly pitted than they are in the epidermal cells (fig. 143, 2).

The third layer is composed of parenchymatous cells with thin walls, which are not pitted; this layer is very difficult to see.

The fourth layer consists of irregularly shaped parenchymatous cells with large intercellular spaces and coarsely pitted walls; it is best seen in a subsequent preparation, in which it is

FIG. 143.

1. Epidermis of pericarp, surface view; *t*, pits on inner wall.
2. Hypoderma of pericarp.
3. Middle layer of pericarp (4 in fig. 10), usual form of cell; 4, unusual form of the same cells.
5. Transverse cells of pericarp, surface view.
6. Cells of the inner epidermis of pericarp, surface view.
7. Cells of seed coats, surface view.
8. Cells of nucellus (after treatment with potash).
9. Cells of epidermis of endosperm (aleurone layer).
10. Transverse section of the pericarp, seed coats, and part of the endosperm of the grain; 1, epidermis; 2, hypoderma; 4, middle layer; 5, transverse cells; 6, inner epidermis of pericarp; 7, seed coat; 8, nucellus; 9, aleurone layer; 10, endosperm.
11. Hair from apex of fruit.
12. Stoma from apex of fruit.
13. Section through the furrow; *b*, vascular bundle; *s*, seed coat.



generally found adhering to the upper surface of the fifth layer ; in places this layer is interrupted (fig. 143, 3 and 4).

The fifth or sclerenchymatous layer. This layer is usually very conspicuous in chloral hydrate ; the cells are elongated transversely to the epidermal cells ; their walls are thickened and conspicuously pitted ; the layer is continuous, and exhibits but few and small intercellular spaces (fig. 143, 5).

The sixth layer (or tubular cells) is often difficult to find, and is better seen in a maceration preparation. The cells are narrow tubes, between which are often large spaces ; the walls are pitted where they adjoin other similar cells. They are arranged transversely to the sclerenchymatous layer (fig. 143, 6).

The seventh layer comprises the seed coats ; it is pale yellow in colour, and is composed of two layers of elongated thin-walled parenchymatous cells crossing one another at right angles (fig. 143, 7).

The eighth layer, or nucellus, is difficult to find ; it is best examined in sections that have been warmed in chloral hydrate (fig. 143, 8).

The ninth layer is the aleurone layer ; the cells are thick-walled, nearly isodiametric, and they are filled with densely packed aleurone grains (fig. 143, 9).

The *endosperm* consists of large thin-walled cells filled with starch grains (fig. 143, 10, 10).

Maceration Preparations.—In addition to the sections and surface preparations it is very desirable to separate the layers of cells as much as possible from one another, and to scrutinise and identify them. For this purpose the following method is an excellent one ; although it requires considerable time, it has the advantage of allowing the tissues to be separated without causing any swelling or other appreciable alteration in the walls. It consists in simply crushing the grains and allowing them to stand, covered with water, in a warm place (20° to 40° C.) ; putrefactive decomposition sets in, and after a few days the layers can be separated by teasing ; they may be examined in dilute glycerin. Examine carefully in this preparation the hairs. They vary considerably in length ; many are between 200 and 500 μ long, but occasionally they are still longer. The walls are thick and the lumen narrow ; towards the apex it becomes gradually narrower, towards the base gradually larger, but at the base itself it enlarges rather

abruptly, and here the wall is distinctly pitted. The diameter at the base is about 18 to 24 μ . In most of the hairs the wall is as thick as, or thicker than, the cavity of the cell.

Having thus thoroughly studied the anatomy of the grain, the student may proceed to study the powder, which should be prepared from dried grains and passed through a 60 or 80 sieve, taking care that the pericarp of the fruit, or part of it at least, passes through the sieve.

The starch may be examined in water as usual and the minute aleurone grains detected with picric acid. The epidermis and aleurone layer can be well seen after soaking in dilute glycerin, but for the examination of the other fragments of pericarp chloral hydrate is the best mountant; allow it to stand until the starch is gelatinised, or effect this by gently warming. The sclerenchymatous cells are generally particularly conspicuous, and to this layer the seed coats and nucellus adhere on the under side, while the upper often bears the interrupted layer of parenchyma (layer 4). The hairs are fairly numerous and readily identified. Most of the tissue found in the examination of the grain may be recognised with facility.

Finely sifted flour consists to a large extent of starch, but it always contains aleurone grains and also fragments of the pericarp (bran). The more efficient the sifting of the flour the fewer and smaller will these fragments be. They are, however, important adjuncts in identifying the flour, and must be collected and examined.

They are best collected as follows:

Mix 5 grammes of flour with 50 c.c. of water, add about 2 c.c. of hydrochloric acid (sp. gr. 1.16), and bring the mixture to the boiling-point; boil gently for five to ten minutes. Cool and add sufficient solution of potash to make the liquid slightly alkaline; it will then become nearly clear. Divide this liquid among the tubes of a centrifuge,¹ and separate the deposit. Collect it in one tube, wash once with water; mount slides of this deposit in water, dilute glycerin, and chloral hydrate respectively. Numerous hairs will be found, as well as small fragments of the pericarp, seed coats, &c., together with various impurities that may be present. The tissues derived from wheat can easily be examined and identified; those foreign to wheat can readily be detected.

¹ If a centrifuge is not available, subsidence in a urine glass may be resorted to.

Diagnostic Characters of other Flours

Vogl¹ gives a useful key for the identification of the various flours from which the following details are taken.

Starch Grains

A. Large grains and very small grains, with but few of intermediate size. Large grains simple, flattened; in side view elliptical or lens-shaped; in surface view circular.

Wheat. Large grains 36 to 39 μ in diameter, isolated grains attaining 45 μ ; in surface view circular or slightly reniform; at most a grain here and there with distinct striations and a cleft or stellate hilum.

Rye. Large grains 36 to 47 μ in diameter, isolated grains attaining 52 μ ; often with very distinct striations and cleft or stellate hilum.

Barley. Large grains 18 to 30 μ , mostly 21 to 28 μ in diameter. In surface view less regularly circular than wheat; often a little irregular in outline, exhibiting depressions or protuberances, broadly reniform or short bean-shaped, or with three or four rounded angles.

B. Large and small spheroidal compound grains, isolated components of the same, and also simple grains.

Oat. Component grains 3 to 7 μ in diameter, angular or with rounded angles; simple grains (3 to 7 μ), associated with very small compound grains with 2 or 3 components. Shape variable; particularly noticeable are crescent-shaped, pointed elliptical and lemon-shaped grains.

Rice. Component grains angular, or with slightly rounded angles; simple grains polyhedral, mostly exhibiting in surface view 5 to 6 sides, almost regular and tolerably uniform, 3 to 9 μ in diameter (mostly 6 μ); most grains exhibiting a conspicuous cavity at the hilum.

¹ *Nahrungs- und Genussmittel*, p. 153.

C. Simple polygonal grains with sharp or rounded angles, together with some rounded grains.

Maize. Grains 6 to 21 μ , mostly 12 to 18 μ , in diameter; sharply polygonal, some with rounded angles, others rounded; often with large hilum or stellate cavity. The grains are sometimes cemented into groups by means of a thin layer of protoplasm, which gives place to a number of minute granules when treated with caustic potash.

Millet. Grains 4 to 12 μ , sometimes 15 μ , seldom showing an angular or stellate cavity; a few smaller, rounded, ovoid, or spindle-shaped. Mounted in water, there is no distinct layer of protoplasm visible.

Buckwheat. Grains 6 to 12 μ , at most 15 to 18 μ , the majority 9 to 12 μ ; sides often concave, less often sharply and uniformly polygonal; some of the groups of cells have remarkable shapes, elongated, club-shaped, curved, &c. Mounted in water there is no distinct layer of protoplasm visible.

Fragments of Tissue

A. Remains of the paleæ present, with characteristic epidermis.

Barley or Oat.

B. Fragments and tissues of the pericarp exhibit the following characters:

(a) Epidermis.

Wheat. Cells with 4 to 6 straight walls, the lateral walls exhibit fairly regular thickening.

Rye. Cells similar, but the lateral walls; exhibit irregular thickening.

Barley. Cells thin-walled, exhibiting at most indications of thickening; stomata and hairs present (see under *b*).

Oat. Cells thin-walled, with hairs (see under *b*).

Maize. Cells with sinuous lateral walls; length up to 180 μ ; width, 15 to 30 μ ; coarsely pitted, thick- and thin-walled.

Millet. Cells with sinuous lateral walls, 30 to 120 μ long, 14 to 30 μ wide.

Rice. Cells with sinuous transverse walls, 60 to 75 μ long, 7.4 to 24 μ wide ; the sinuosities mostly on the short walls.

(b) **Hairs.**

Wheat. Up to 1 mm. long ; the wall as wide as or wider than the lumen ; often bent above the basal part.

Rye. Similar to wheat, but the wall narrower than the lumen and the hairs not bent.

Barley. Thin-walled, conical, or trumpet-shaped, 30 to 180 μ long, 9 to 21 μ wide.

Oat. Usually two or three together, straight, very long, and gradually tapering.

(c) **Transverse Cells.**

Wheat. Single layer of thick-walled cells with almost straight longitudinal walls, which are rather regularly pitted and exhibit no intercellular spaces.

Rye. Similar, but the ends are often rounded and strongly thickened ; 3- to 4-sided intercellular spaces.

Barley. Two rows of thin-walled cells with abundant intercellular spaces.

Oat. Single layer of cells, their long axes often oblique to the epidermal cells.

Rice. Cells narrow ; strongly tangentially elongated, not always closely abutting on one another.

(d) **Tubular Cells.**

Wheat, rye, barley. Cells often thick-walled, 15 to 30 μ wide, often with spaces between or only few present.

Rice, millet, maize. Cells very numerous, close together, mostly 3 to 5 μ wide (in maize two rows crossing one another).

C. Cells of aleurone layer exhibit the following characters.

Wheat. Cells in a single row, very thick-walled ; in section almost quadratic.

Rye, oat, maize. Cells in a single row, thick-walled ; in section radially elongated.

Rice, millet. Cells tend towards tangential elongation ; relatively thin-walled, not collenchymatous.

Buckwheat. Cells similar, but rather thick-walled and collenchymatous.

Barley. Two or three rows of cells.

Examination and Identification of Powdered Fruits.—The methods adopted for the examination and identification of powdered fruits are the same as those for powdered seeds. It is frequently very difficult to distinguish one from the other, but the presence of empty parenchymatous cells, of vessels in any abundance, or of fragments of epidermis with half obliterated stomata usually indicates a powdered fruit.

SECTION XI

RHIZOMES

INTRODUCTION

IN its widest signification the term 'rhizome' includes all hypogæic or epigæic stem formations which differ from aerial stems in their shape, appearance, size, duration, structure, &c. They are usually thickened and horizontal or nearly so, but they may be slender and may assume temporarily or permanently an oblique or erect position. Sometimes the branches only of the rhizome ascend and produce leafy and flowery stems, while the rhizome itself continues its growth. But more frequently the axis itself curves upwards and elongates into an aerial stem. This stem is annual, and when its period of growth is completed it perishes. This would cut short the life of the rhizome were it not for the fact that a bud in the axil of one of the cataphyllary leaves borne by the rhizome and its branches develops and continues the life of the rhizome, forming simultaneously a sympodial branch system.

The structure of the typical horizontal rhizome, such as that of *podophyllum*, *arnica*, &c., is analogous to that of aerial stems.

The stele is separated from the cortex by an endodermis, which is often easily discernible under the microscope. The tegumentary tissue is usually cork, the epidermis having in most cases been thrown off. The tissue which has been designated 'outer bark' (see p. 139) is comparatively seldom produced. The cortex is often largely developed, and is obliquely traversed by leaf traces passing into the cataphyllary leaves. In the stele of dicotyledonous rhizomes the wood bundles may be arranged in a close or diffuse ring; here also the parenchyma is often largely developed and filled with reserve material.

Most of the official rhizomes are obtained from monocotyledonous plants. In these the bundles are closed and no cambium

is formed. Very frequently each bundle is supported by a crescent-shaped mass of sclerenchymatous fibres. In those rhizomes in which the internodes are very closely approximated these bundles often assume an oblique or even nearly transverse direction.

As there is by no means so great a differentiation of the tissues in subterraneous organs as in aerial, the tissues themselves of rhizomes offer fewer diagnostic characters than those of aerial stems, and it becomes necessary to pay very careful attention to the minuter details.

Most rhizomes serve as organs for the storage of reserve materials, and the parenchymatous tissue destined to receive these is therefore largely developed. During the process of pulverisation these cells, being usually thin-walled, are more or less completely broken up, and hence the powder consists largely of the reserve material which they contained and of the *débris* of the cell walls. Concerning the reserve materials sufficient has already been said in the preceding chapters, and the student will be acquainted with the importance and means of distinguishing starch, calcium oxalate, &c., as well as the various forms of secretory tissue.

Particular attention must be paid to the nature of the walls of the parenchymatous cells, their thickness, the size and distribution of the pores, &c., as by these means the parenchyma of one drug may often be distinguished from that of another. The vessels and fibres must be isolated, and their shape, colour, thickness of wall, and nature of the pores carefully studied.

The examination of the powdered drug is best conducted first in water or dilute glycerin, in which the colour of the tissues present, the details of the cell walls, of starch, &c., may be well observed. Boiling with dilute solution of potash which is subsequently washed out with water often clears the powder from cell contents, and leaves the cell walls practically unaltered. Digestion with chloral hydrate often answers the same purpose, the powder being separated from the liquid by a centrifuge. Dissociation of the elements, which is often necessary, may be effected by maceration with potassium chlorate and nitric acid, while for very starchy rhizomes boiling with dilute hydrochloric acid followed by dilute potash, as detailed under wheat flour, yields excellent results, the details of the vessels and fibres being usually very clear.

Arnica Rhizome

Source.—The rhizome of *Arnica montana*, Linn.

Examination of Sections.—Select two or three pieces of arnica rhizome, breaking them to see that they are sound. Soak them twelve hours in water, and then expose them to the air until they assume a tough condition in which they are suitable for cutting. Cut transverse sections, and transfer them to spirit.

Mount and examine one in chloral hydrate. Observe the circle of irregular wood bundles; within the circle is the pith;

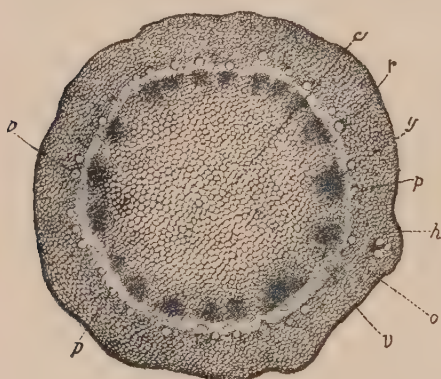


FIG. 144.—Arnica Rhizome, transverse section. c, pith; o, cortex; p, oleoresin ducts; r, medullary rays; v, bast; y, wood bundles. $\times 10$. (Berg.)

beyond it are the bast ring, cortex, and tegumentary tissue (fig. 144). In order that the last-named three tissues may be correctly described it is necessary that the position of the endodermis—which is the innermost layer of cortical cells, and consequently separates the cortex from the bast ring—should be determined. This is possible with arnica, and also

with many other, but not all, rhizomes.

In arnica rhizome the endodermis can usually be found without special treatment. Observe between the tegumentary tissue and the wood bundles a number of large schizogenous oleoresin ducts arranged in a circle. The endodermis is just within this circle; indeed it often curves round the inner margins of the ducts, thereby assuming a sinuous course. The cells of which it is composed are narrow, tangentially elongated, and characterised by distinct suberisation and lignification in the centre of the radial walls (compare *Lobelia Stem*, p. 79). This lignified and suberised portion is strongly refractive, and hence usually easily visible. Should it not be apparent, it can be made more conspicuous by staining it with phloroglucin and hydrochloric acid.

Having determined the position of the endodermis, make a diagrammatic sketch of the section.

Examine now in succession the tissues of which the rhizome consists, commencing with the outside.

(1) **Tegumentary Tissue.**—If the cells of which this is composed are not easily seen in chloral hydrate, and this is often the case, mount a fresh section in solution of potash, warm it gently, and cool; they will then be distinctly visible; the tissue consists of several rows of cork cells, with thin brown walls.

Examine a very thin section in water; these cells (or at least some of them) have brown amorphous contents. Irrigate the section with dilute solution of ferric chloride; the brown changes to greenish black (tannin reaction).

(2) **Cortex.**—The tissue from the phellogen up to and including the endodermis is cortex. Careful examination will show that no secondary cortex (pheloderm) has been formed. The cortical parenchyma consists of cells with slightly thickened pitted walls; most of the cells are somewhat tangentially elongated, and near the phellogen are often of collenchymatous nature. Near the endodermis there is a diffused circle of large oleoresin ducts of obviously schizogenous origin. These ducts generally exhibit a well-defined tapetal layer, from

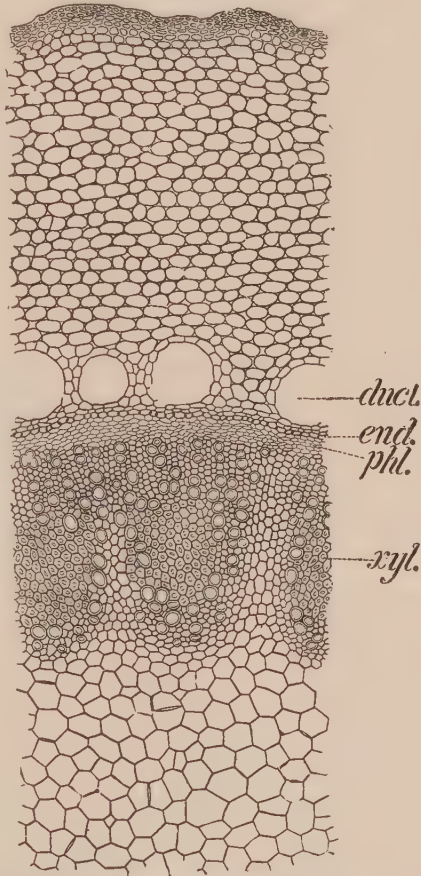


FIG. 145.—*Arnica* Rhizome, transverse section. *end.*, endodermis; *phl.*, phloem; *xyl.*, xylem. $\times 80$. (Partly after Berg.)

the inner cell wall of which little gelatinous papillæ project into the cavity of the duct. These papillæ are the remains of the resinogenous layer of the cell wall ; in this layer the oleoresin is formed, and from it the secretion is discharged into the cavity of the duct (Tschirch). Similar but smaller ducts may also be observed in the cortex, nearer the cork, accompanying in pairs small fibro-vascular bundles (leaf traces).

The cells of the cortex contain small granules. Some of these stain yellow with iodine, and are doubtless granular remains of the protoplasm. Here and there large amorphous or indistinctly crystalline lumps of inulin are to be seen (compare the characters of this substance detailed under *Taraxacum* Root). Tincture of alkanna shows the presence of but little oil. With concentrated sulphuric acid fairly abundant

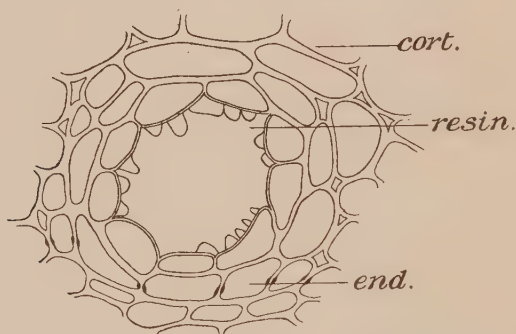


FIG. 146.—*Arnica* Rhizome, transverse section of oleoresin duct, showing the resinogenous layer. $\times 250$.

acicular crystals of calcium sulphate are obtained, indicating the presence of notable quantities of this metal.

The bast ring is narrow, and consists of small elements that do not call for special note. In the wood bundles the centre is generally occupied by a mass of wood fibres, often of considerable extent. The pith is composed of rather large parenchymatous cells, with intercellular spaces ; in these cells lumps of inulin may often be found.

Ginger

Source.—The rhizome of *Zingiber officinale*, Roscoe.

Examination of the Rhizome.—For examination select a piece of unbleached Jamaica ginger from which the cork has

been removed by scraping. Break the rhizome transversely between two of the branches, smooth the broken, transverse surface with a knife, and examine.

Observe the large stele, which is surrounded by a distinct line usually designated the endodermis; the cortex is comparatively narrow. Throughout both stele and cortex there are scattered dark bundles and pale yellowish brown secretion cells.

Transverse Sections.—From the dry transverse surface cut several sections, taking care to cut from the stele as well as from the cortex, and to include therefore all tissues from the margin to the centre. Transfer them to alcohol, bearing in mind, of course, that the oleoresin will be dissolved; direct attention at first to the structure, and leave the cell contents to be studied later with due precautions.



FIG. 147.—Ginger Rhizome. *A*, transverse section $\times 3$; *B*, radial section, natural size. *o*, cortex; *v*, endodermis; *b*, stele; *h*, cork (or outer portion of cortex). (Berg.)

Mount a section in water and examine under the low power; there is so much starch present as to obscure the structure.

Mount another in chloral hydrate and gelatinise the starch by warming. The structure becomes more distinct. The cortex is free from tegumentary tissue of any kind (it has been removed by peeling). It consists of thin-walled parenchymatous cells, with intercellular spaces. This tissue contains scattered oil cells, and is traversed by fibro-vascular bundles (leaf traces), which are cut transversely, or at least only slightly obliquely.

The inner limit of the cortex (endodermis) cannot be easily identified. Observe towards the interior of the rhizome a pale brownish circle (visible under a lens). On the inner (concave) side of this line a number of fibro-vascular bundles can be

seen ; the structure of the line itself is not very distinct, the cells being more or less collapsed. Mount another section in

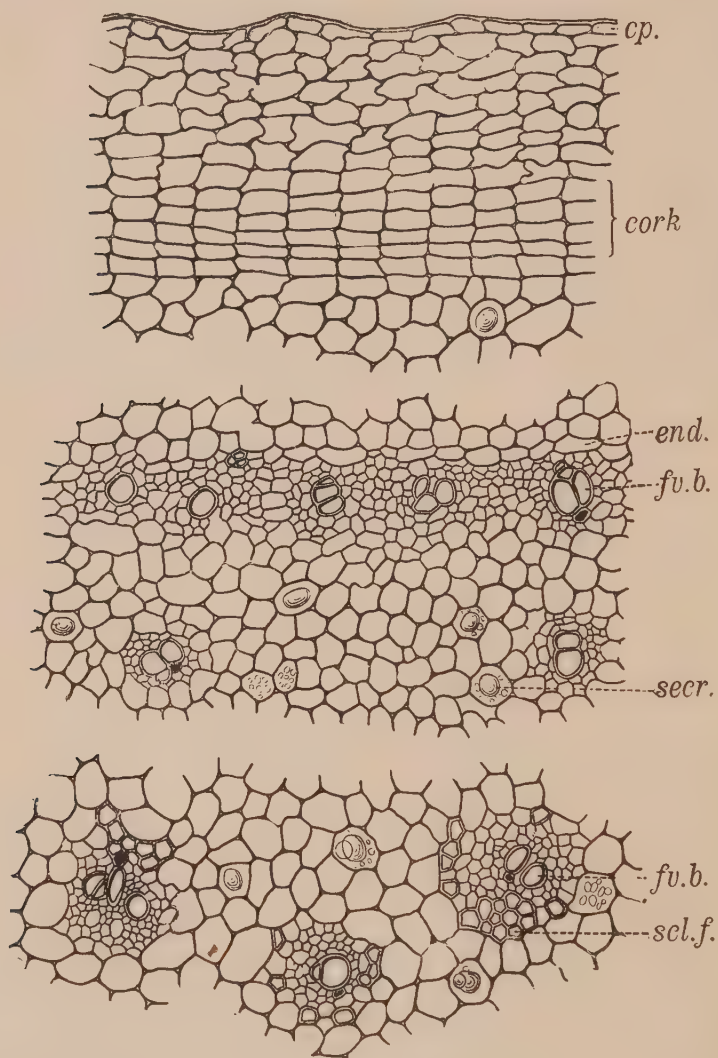


FIG. 148.—Ginger Rhizome, transverse section. *end.*, endodermis; *ep.*, epidermis, which together with the subjacent cork is often removed by scraping; *fv.b.*, fibro-vascular bundle; *scl.f.*, sclerenchymatous fibres; *secr.*, secretion (oleoresin) cells. (Tschirch and Oesterle.)

concentrated (25 to 50 per cent.) solution of potash ; warm gently, not quite to boiling. The walls of the collapsed cells

expand, and the endodermis can be recognised by its strongly refractive radial walls. Within the endodermis, between it and the circle of bundles, are two (or three) rows of tangentially elongated parenchymatous cells, but the radial walls of these cells are not strongly refractive. Wash out the solution of potash with water; irrigate with concentrated sulphuric acid. The radial walls of the endodermis do not dissolve; they are suberised.

The bundles immediately within the endodermis are tangentially elongated, and abut so closely upon one another as to form an almost continuous circle. The remainder of the stele consists of thin-walled parenchymatous cells, with scattered oil cells, and more or less rounded fibro-vascular bundles.

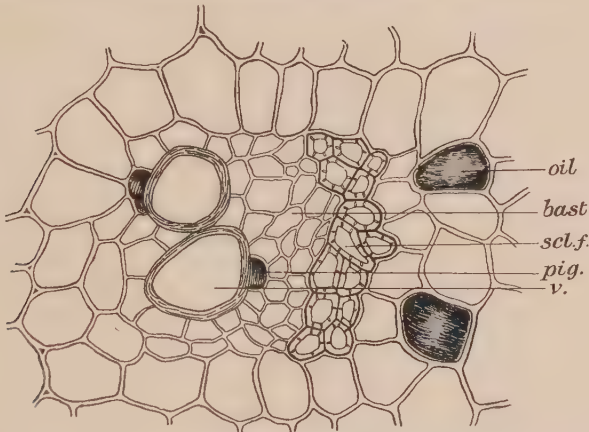


FIG. 149.—Ginger Rhizome, transverse section, through a bundle, with neighbouring parenchyma; *oil*, oleoresin cells; *pig.*, pigment cells; *scl.f.*, sclerenchymatous fibres; *v.*, vessel. (Vogl.)

Examine these bundles more carefully. They are collateral, and consist of a few vessels, mostly of considerable size, abutting upon bast tissue, the whole supported or surrounded by fibres with thickened walls. In some of the bundles one or sometimes two small cells filled with a brown homogeneous amorphous substance can be seen adjoining one of the vessels.

Cut and examine longitudinal sections of the rhizome, selecting those that pass through the bundles. The parenchymatous cells are axially elongated. The vessels are spiral, reticulated or scalariform (or intermediate between these). The

fibres have rather numerous elongated or cleft pits arranged in a left ascending spiral.

The brown substance in the small cells above referred to is soluble in solution of potash and in chloral hydrate, and may therefore escape observation. Mount a fresh section in water, and remove this by filter paper; drop on concentrated hydrochloric acid, and gently warm to expel air bubbles. The secretion cells are now very conspicuous; they are narrow elongated tubular cells, which, however, are not continuous, and therefore are not seen in the transverse section of every bundle. These secretion cells are characteristic of *Zingiberæ*.

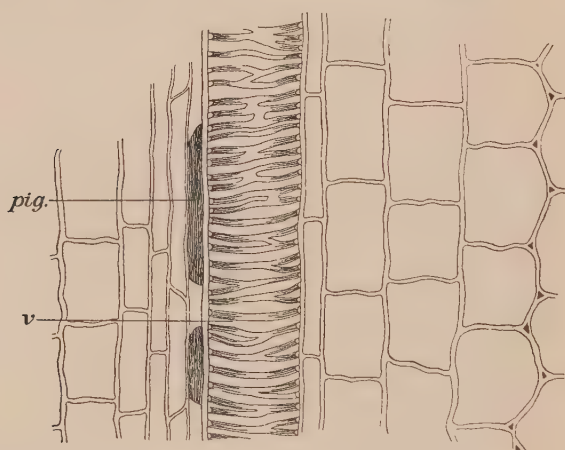


FIG. 150.—Ginger Rhizome, radial section through a portion of bundle.
pig., pigment cells; *v*, vessel. (Vogl.)

Isolate some of the fibres and vessels by maceration with potassium chlorate and nitric acid, and examine. The fibres vary both in length and shape. Some taper at the ends, others are rounded, others again are square. Sometimes they exhibit thin transverse divisions. The pits are not easily seen after the treatment with nitric acid; they are better studied in longitudinal section (see above). In this preparation numerous oil cells containing the oleoresin may be found.

Examine next the starch grains. Many of them are sack-shaped, ovoid or elongated ovoid, flattened and more or less pointed at one end, but rounded at the other, the hilum being close to the apex of the pointed extremity and so eccentric as to be almost invisible; the smaller grains are often nearly circular.

The striations are distinctly visible, especially in the larger grains, when carefully illuminated.

The grains vary mostly from 20 to 30 μ in length, but some measure as much as 30 to 40 μ , while occasionally 45 to 50 μ is reached; the thickness is generally from 5 to 10 μ .

Proceed now to determine the distribution of the oleoresin cells. Cut a moderately thick transverse section, mount in glycerin, or dilute glycerin, and gently boil to expel air and gelatinise starch. Examine under a low power; the oleoresin cells are scattered throughout the cortex and stele: they contain a pale yellow oleoresin.

A section similarly treated with solution of caustic potash also shows the oleoresin cells, but they will now contain a deep reddish-brown liquid in which globules of oil are visible, the resin having combined with the alkali to form a dark-coloured compound in which the globules of oil remain suspended.

Ginger Powder

Examination of the Powder.—The student should now examine powdered ginger with the object of identifying in it all those structural elements and cell contents that have been observed in the rhizome.

Moisten a little powdered ginger with water; allow it to stand a few hours, and then examine. The preparation contains abundance of starch grains; examine these carefully, and compare with the starch grains of the rhizome; they often appear granular from adhering particles of proteid matter, &c., from which they can be partially freed by mounting in very dilute (0.1 per cent.) solution of potash. Most of the cells with yellow or brownish oleoresin are destroyed by the grinding, but a few remain intact. The sclerenchymatous fibres are pale yellowish in colour.

To examine the sclerenchymatous elements present, it is desirable to get rid of the starch, and thus concentrate the vessels, fibres, &c., into a smaller compass. This may be done as follows:

Mix 5 grams of the powdered ginger with 50 c.c. of water and add 2 c.c. of the official hydrochloric acid (sp. gr. 1.16). Raise to the boiling-point, and boil gently for ten minutes. Then centrifuge for two minutes, which suffices to separate all the

sclerenchymatous tissue and most of the parenchyma. Wash once with water, separating again by the centrifuge. Stir the residue in a few cubic centimetres of solution of chloral hydrate and again separate by the centrifuge, pour off the chloral hydrate, and examine the deposit either in that medium or in glycerin. If a centrifuge is not at hand, the deposit obtained from the acid liquid may be washed with very dilute (about

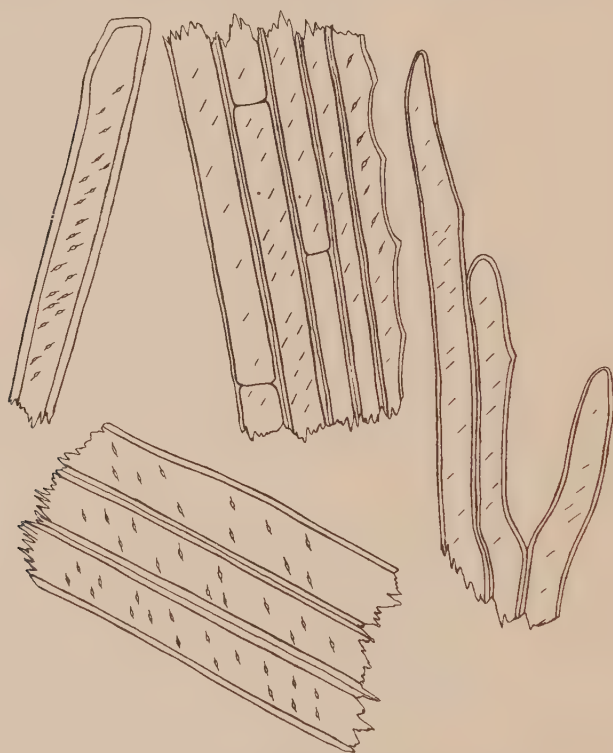


FIG. 151.—Ginger Rhizome, fragments of sclerenchymatous fibres in the powder. $\times 200$.

·5 to 1 per cent.) solution of potash, and finally once with water.

The deposit will be free from starch, oleoresin, and proteid matter; it will consist of the sclerenchymatous fibres, the vessels, and the *débris* of the parenchymatous tissue, all of which may be examined in this preparation.

The fibres exhibit their shape and left-spiral pits; the

vessels are mostly reticulate, and vary from 30 to 70 μ in diameter; here and there the brown secretion cells that abut on them may be detected; the parenchymatous cells have very thin walls, those of the oleoresin cells being suberised; the latter may be detected by staining with Soudan red.

Galangal Rhizome

Source.—The rhizome of *Alpinia officinarum*, Hance.

Preparation and Examination.—Galangal rhizome may be prepared and examined by the methods recommended for

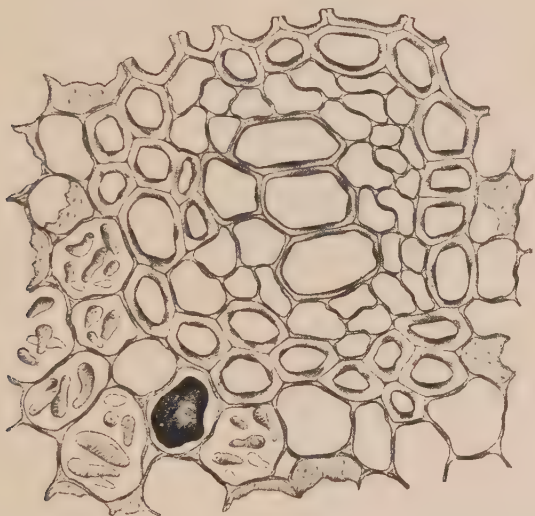


FIG. 152.—Galangal Rhizome, transverse section of a fibro-vascular bundle, with adjacent parenchyma; some of the cells contain starch, in one a dark mass of oleoresin. (Moeller.)

ginger, which it resembles in structure. Differences are to be found in the following particulars :

- (a) The starch grains are rather larger than those of ginger ; many are club-shaped, others are reniform, some exhibiting irregular protuberances. They are not flattened, as scitamineaceous starches usually are, and are distinctly striated ;

- (b) The parenchymatous cells have thicker walls and are brownish in colour; the secretion cells are numerous, and contain a dark reddish-brown oleoresin.
- (c) The bundles are more numerous than those of ginger, and therefore the powder contains more bast fibres and vessels.



FIG. 153.—Galangal Rhizome, fragment from the powder. *am*, starch; *bf*, bast fibres; *g*, vessel; *p*, thick-walled parenchymatous cells. $\times 160$. (Moeller.)

Turmeric

Source.—The rhizome of *Curcuma longa*, Linn.

Turmeric resembles ginger and galangal in structure, but differs from both in the absence of bast fibres. It is further characterised by the yellow colouring matter, which, however, is no longer restricted to particular cells, as it is in the fresh rhizome, but is distributed throughout the drug. This change is due to the scalding to which the rhizome is subjected, and this also causes the gelatinisation of most of the starch grains. Many of the parenchymatous cells, therefore, contain irregular yellow masses of gelatinised starch. Although most of the

starch is swollen, here and there grains may be found that still exhibit the characteristic scitamineous shape.

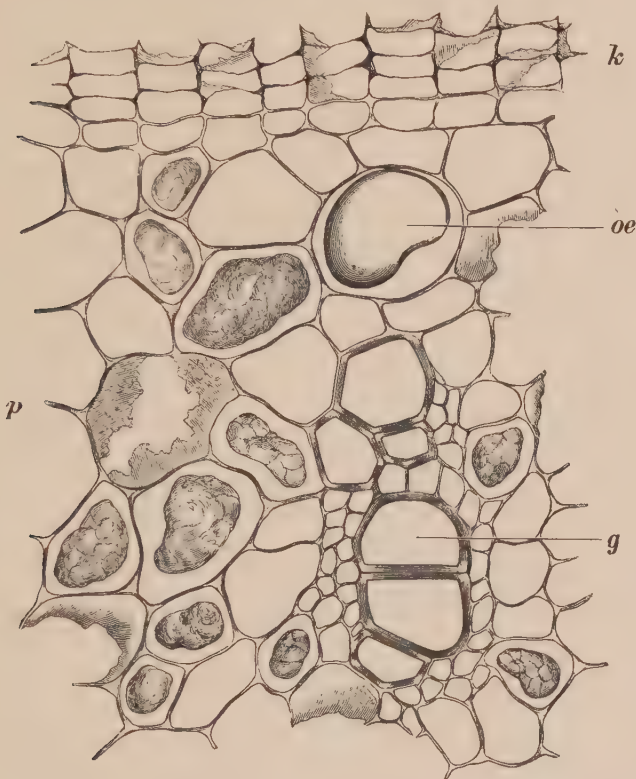


FIG. 154.—Turmeric Rhizome, transverse section. *g*, vessel; *k*, cork; *oe*, oleoresin; *p*, parenchyma of wood, containing masses of gelatinised starch. (Moeller.)

The yellow colour is changed to deep red by sulphuric acid, either concentrated or diluted with an equal volume of water or alcohol; in the latter case the red substance produced dissolves. This reaction, which is due to the action of the sulphuric acid upon the curcumin of the rhizome, forms a useful means of detecting powdered turmeric.

SECTION XII

ROOTS

INTRODUCTION

NEARLY all the roots used in medicine are derived from dicotyledonous plants, and hence bear, as might be anticipated, so close a resemblance in structure to dicotyledonous rhizomes that the methods of treatment, &c., that have been described for rhizomes are equally applicable to roots. It must be borne in mind that many drugs commonly called roots consist either entirely (rhubarb) or partially (liquorice) of rhizome, and that some roots pass gradually towards the upper part into rhizome (taraxacum), so that a mechanical separation of rhizome from root becomes impossible. While very young roots can without difficulty be distinguished from very young rhizomes by the relative position of the bast and wood bundles, this is by no means the case with older ones, in which this difference has become so obscured as, with few exceptions, to elude detection. Rhizomes frequently bear cataphyllary leaves, the scars of which are often visible in the dry drug; these leaves are connected with the stele of the rhizome by meristeles that pursue a more or less oblique course through the cortex, and hence are often visible in a transverse section. Roots, on the other hand, are free from such leaf traces. Rhizomes further often contain a distinct pith from which roots are free, but it must be remembered that many roots contain a central parenchymatous tissue practically indistinguishable from the pith of stems.

Monocotyledonous roots differ essentially from dicotyledonous in containing closed instead of open bundles; they therefore usually exhibit clearly their primary structure.

Belladonna Root

Source.—The root of *Atropa Belladonna*, Linn.

Examination.—Select a small piece of starchy belladonna root about $\frac{1}{4}$ inch in diameter; expose it to a moist atmosphere for a few hours. Cut transverse sections, and immerse them in alcohol. Examine one in dilute glycerin. There is an abundance of starch in the cells; examine this later.

Clear a section by solution of potash (or by chloral hydrate), warming, if necessary, to exhibit the cork cells. The cork consists of three or four rows of tangentially elongated tabular cells, on the outside of which there is often a little granular matter (particles of earth) to be seen.

Determine the position of the cambium, which is easily seen.

The cortex (which is developed from the endodermis or pericycle, and is, therefore, botanically a phelloderm or secondary cortex) consists of parenchymatous cells with rather thick walls and large pits; the walls have been swollen a little by the action of the caustic alkali, especially if the preparation has been warmed. There are numerous intercellular spaces. Near the cork the cells exhibit marked tangential elongation; towards the cambium they become more nearly isodiametric.

In the bast ring, which is not sharply delimited towards the outside, observe radially arranged groups of sieve tissue; they may be recognised by the very small diameter of the cells, their thinner walls (after warming), and the absence of pits, but sieve plates are not easy to detect. There are no bast fibres.

The wood within the cambium consists largely of thin-walled parenchyma, throughout which radially elongated groups of vessels are distributed. The centre of the root is occupied by the primary wood.

The bundles contain a few (often 3 to 8) vessels, accompanied by smaller elements the nature of which is not so evident (compare radial section).

Most of the parenchymatous cells contain starch grains, but here and there a cell can be seen filled with a dark granular, often almost black, substance. Closer examination shows this to be sandy crystals, and the usual tests will indicate it to be calcium oxalate (compare Dulcamara, p. 85).

Examine the starch in water. Most of the grains are com-

pound, and consist of two or three component granules which vary considerably in size. The majority are about $15\ \mu$ in diameter, but some are minute, while others may attain exceptionally as much as $30\ \mu$.

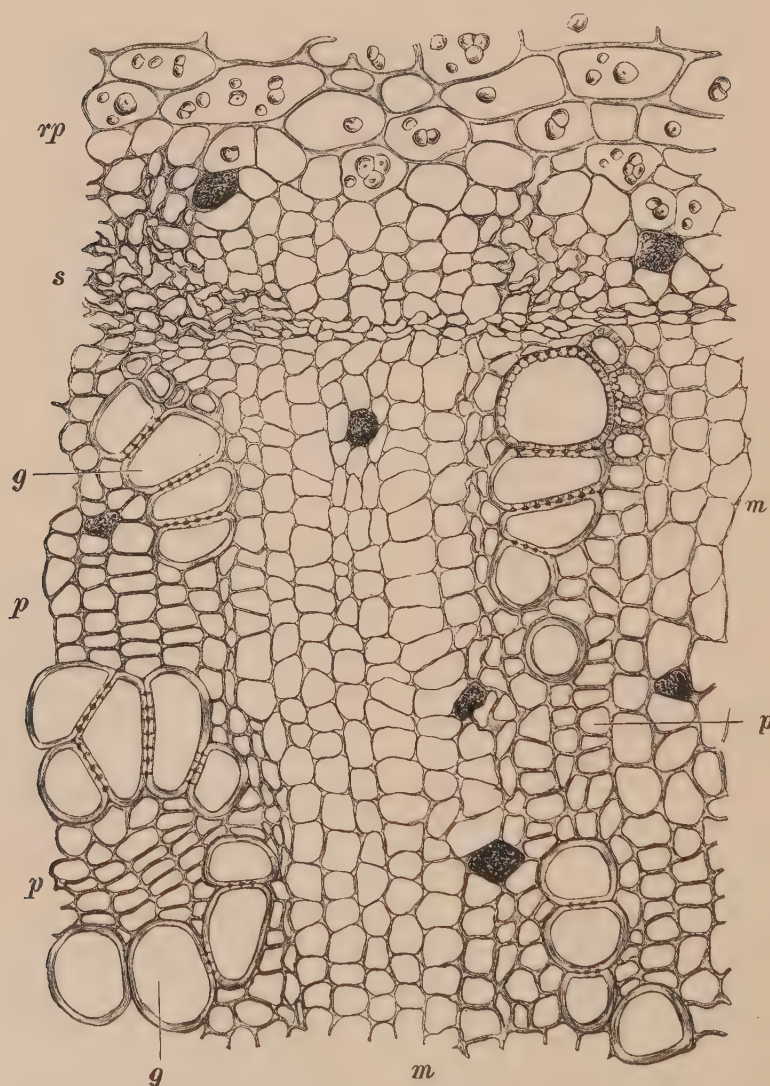


FIG. 155.—Belladonna Root, transverse section. *g*, vessels; *m*, medullary rays; *p*, parenchyma of wood; *rp*, cortical parenchyma; *s*, bast groups, with sieve tubes; the dark contents in some of the cells are sandy crystals of calcium oxalate. (Moeller.)

Prepare now radial sections. The parenchymatous cells are mostly axially elongated. The vessels have pitted walls. The smaller elements associated with the vessels are mostly tracheids passing by a series of intermediate forms into fibres, of which, however, but few typical ones are present. Most of the tracheids are elongated, narrow, with bluntly pointed

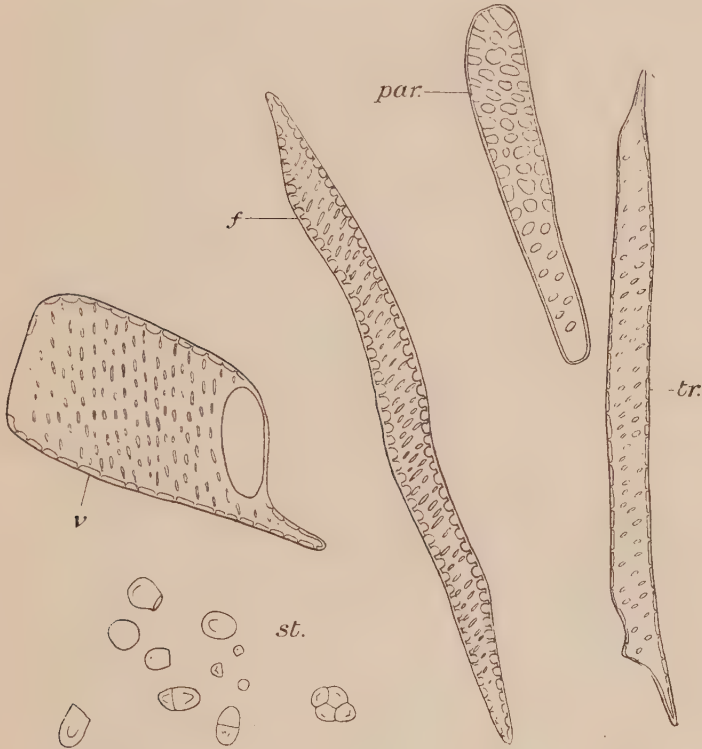


FIG. 156.—Belladonna Root, elements of wood, separated by potassium chlorate and nitric acid. *f*, fibre; *tr.*, tracheid; *par.*, wood parenchyma cell, reticulated at one extremity, pitted at the other; *v*, vessel; *st.*, starch (in water). $\times 200$.

extremities and numerous large rounded or oval pits, often arranged in a left ascending spiral. The latter form of tracheid is intermediate between the typical tracheid and the wood fibre; in the latter the pores are slits and by no means numerous. The cells filled with sandy crystals are conspicuous and are axially elongated.

Marshmallow Root

Source.—The root of *Althæa officinalis*, Linn.

Examine this in the same way as belladonna. Observe especially in the cortical portion, but also in the wood, bast

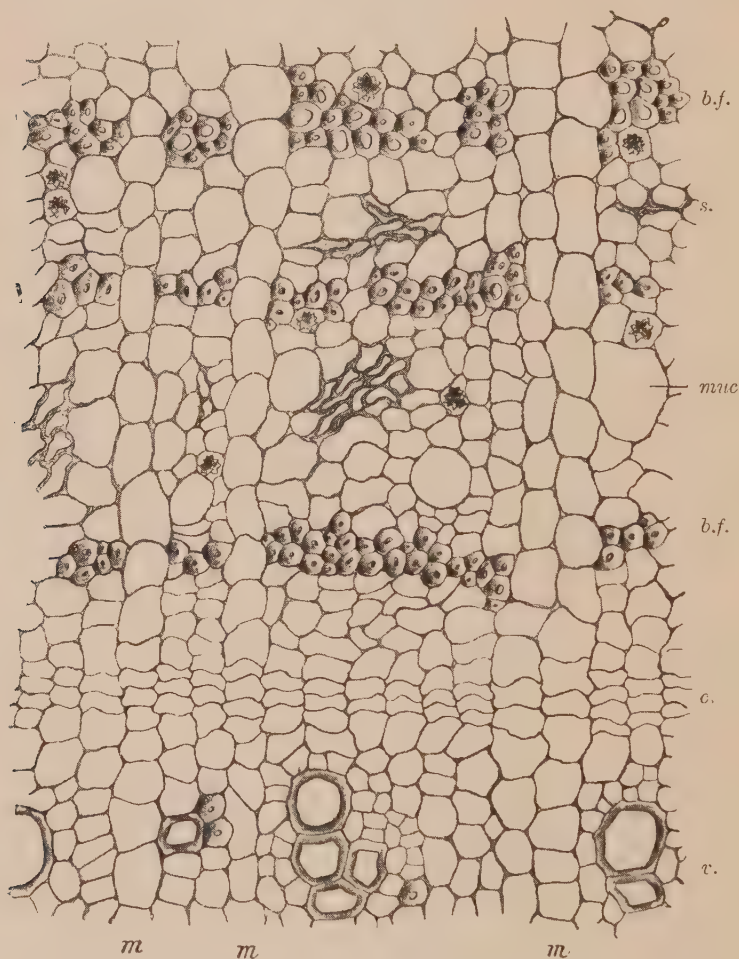


FIG. 157.—Marshmallow Root, transverse section. *b.f.*, bast fibres; *c.*, cambium; *muc.*, mucilage cell; *s.*, sieve tubes; *v.*, vessels; from a section cleared with potash. (Moeller.)

fibres with small lumen and rounded or polygonal outline. The starch grains are about the same size as those of belladonna

root, but they are simple, elongated, ovoid, or reniform in shape. Calcium oxalate is present in the form of rosettes.

In both cortex and wood there are numerous isolated parenchymatous cells filled with a colourless more or less transparent mass (mucilage). This can be stained red with ruthenium red in lead acetate. If a section is cut dry, mounted in alcohol or glycerin, and irrigated with water, the mucilage will swell, exhibit distinct stratification, and finally dissolve.

In this root the shape and length of the bast fibres, the nature of the pits in the walls &c. should be determined by radial sections as well as by isolation.

Dandelion Root

Source.—The root of *Taraxacum officinale*, Wiggers.

Preparation and Examination.—Select for examination some small pieces of dandelion which exhibit a pale interior when broken. Smooth the transverse surface and examine with a lens; observe the small yellowish wood and wide cortex, the latter traversed by numerous narrow, dark, concentric rings (fig. 156). Reject pieces that exhibit a pith in the centre of the wood; they are pieces of rhizome, into which the root imperceptibly passes. Expose suitable pieces to a moist atmosphere until they are ready to cut (about twelve hours). Cut transverse sections, and transfer them to alcohol.

Examine a section in water. The wood consists of yellowish vessels irregularly intermingled with thin-walled parenchyma. The nature of the vessels can be determined, if desired, by longitudinal sections, and by isolating the elements by means of potassium chlorate and nitric acid as usual.

Examine the cortical portion of the section. Many of the parenchymatous cells, or at least some of them, contain colourless amorphous masses of irregular shape. They consist of inulin (see later). Clear the section by gently warming (about 60–80° C.); the inulin dissolves completely without swelling (distinction from starch).

The cortex of the root consists of thin-walled parenchyma

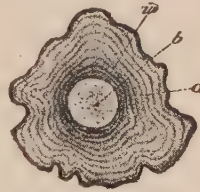


FIG. 158.—Dandelion Root, transverse section. *a*, bark; *b*, wood; *w*, cambium. $\times 4$. (Berg.)

bounded by a thin brown line of tegumentary tissue, which, after warming with potash, is seen to consist of a few rows of cork cells with brown contents.

The concentric rings in the cortex, which are so conspicuous under a lens, are made up of a series of groups of small cells; these groups are separated from one another by a few parenchymatous cells.

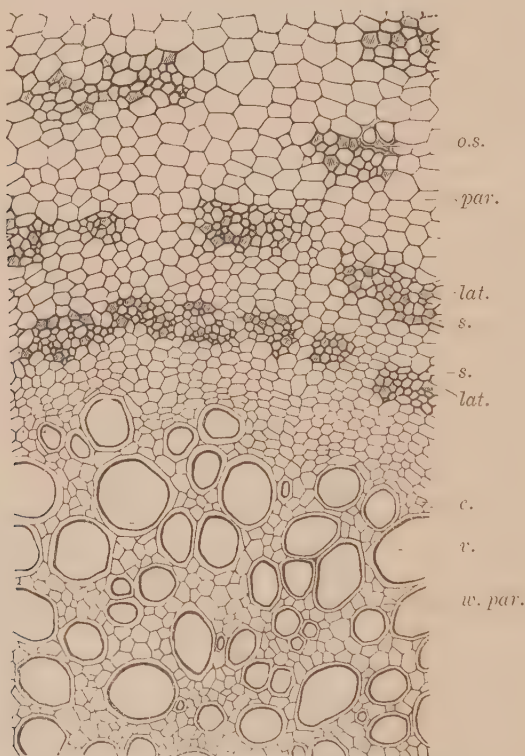


FIG. 159.—Dandelion Root, transverse section. *c.*, cambium; *lat.*, laticiferous vessels; *o.s.*, obliterated sieve tubes; *par.*, parenchyma of cortex; *s.*, sieve tubes; *v.*, vessel; *w. par.*, wood parenchyma. (Tschirch.)

Examine one of these groups closely under the high power. Some of the small cells contain a brownish or nearly black granular substance. These are laticiferous vessels.

Stain a cleared section with corallin-soda. In some at least of the cells that were apparently empty a bright pink mass will be seen. This is a callus plate, and indicates that the element is a sieve tube.

Further elucidate the nature of these elements by preparing tangential sections as follows: Cut off a piece of root about $\frac{1}{4}$ inch long, and shave off longitudinally about one half of the cortex, taking care to keep the plane of the cut surface parallel to the long axis of the root; this is easily done by observing that the dark longitudinal lines (sections of the concentric rings) on the tangential section are kept parallel to one another and do not converge; then with the razor cut tangential sections until one of the concentric rings is reached and passed. Transfer these sections to water; clear as before.

The tissue consists largely of axially elongated parenchymatous cells, through which there runs an anastomosing network of laticiferous vessels with brownish granular contents; they can be seen to have lateral anastomoses with neighbouring vessels.

Stain a cleared section with corallin-soda; the callus plates of the sieve tubes stain pink; the latter are numerous, and their sieve plates are often arranged in rows. With care the course of the sieve tubes may be followed; they accompany the laticiferous vessels.

Digest some tangential sections for a few minutes with solution of potash on a water-bath. The vessels can be teased out and their anastomoses easily seen.

Mount a thin tangential section in water. The chief contents of the parenchymatous cells is inulin; observe it closely. The lumps are colourless, and vary very much in size and shape. Sometimes there is only one in the cell, sometimes several. Not infrequently they exhibit a more or less distinct

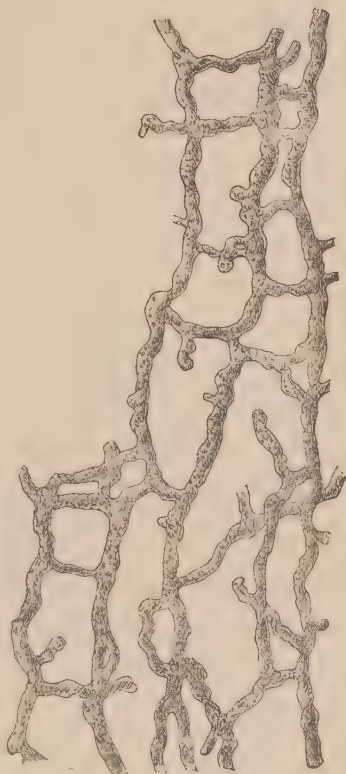


FIG. 160. -Anastomosing laticiferous vessels of Dandelion Root. $\times 140$. (Vogl.)

sphærocrystalline structure ; in other cases the latter is much less perceptible.

Test them with iodine water ; they fail to react. Warm the section very gently (in water) ; they dissolve without swelling.

Chicory

Source.—The root of *Cichorium Intybus*, Linn.

Chicory root closely resembles dandelion root in structure, but it is usually much larger in size and not so easy to examine.

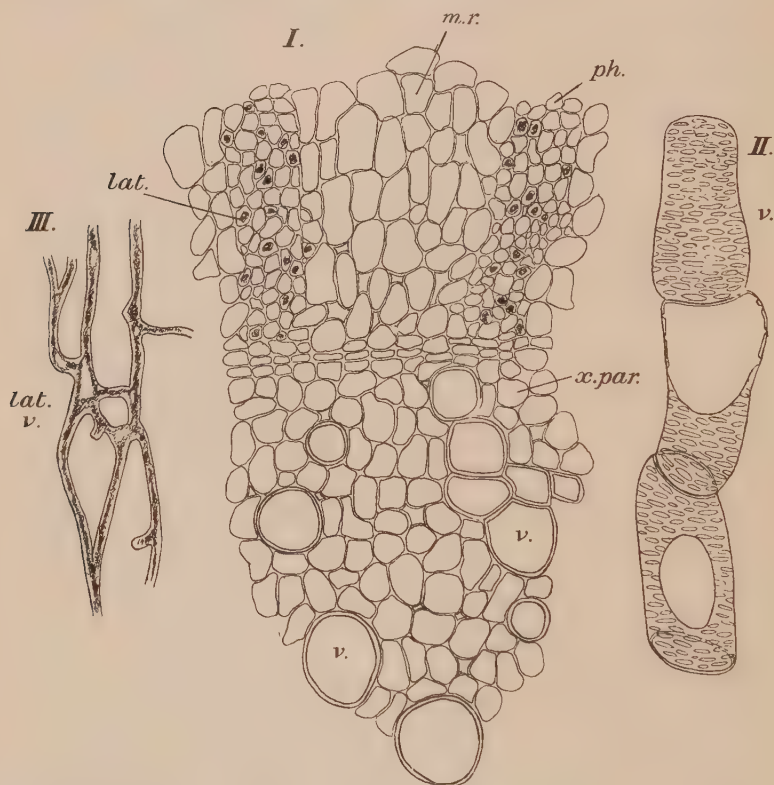


FIG. 161.—Chicory Root. *I.*, transverse section; *lat.*, laticiferous vessel; *m.r.*, medullary ray; *ph.*, phloem; *v.*, vessel; *x.par.*, parenchyma of xylem. *II.*, radial section of vessels. *III.*, laticiferous vessel isolated by maceration with potash. $\times 220$.

Procure from a grocer some raw chicory roots. Prepare them for cutting by soaking for fifteen minutes in water,

and then hardening for three or four hours in alcohol. Cut sections with a razor flooded with alcohol, and transfer them to alcohol. Take care that they include part of the wood as well as of the cortical portion. Transfer one to a slide, mount in water, and clear with chloral hydrate.

Observe in the wood the largely developed thin-walled parenchyma in which large vessels are distributed, mostly in radial lines one or two vessels wide. The cortical portion also consists largely of parenchyma. The bast rays are narrow, and alternate with wide medullary rays. In the former groups of

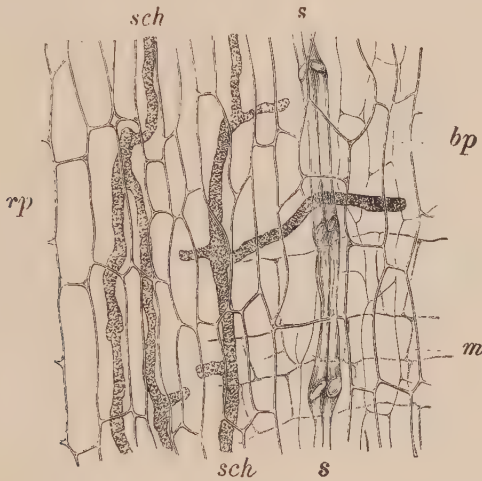


FIG. 162.—Chicory Root, radial section of cortex. *bp*, bast parenchyma; *m*, medullary ray; *rp*, cortical parenchyma; *sch*, laticiferous vessels. $\times 160$. (Moeller.)

sieve tubes and laticiferous vessels alternate with groups of bast parenchyma.

Examine tangential sections of the cortex; observe the anastomosing laticiferous vessels accompanied by narrow sieve tubes with small transverse plates, resembling those of the dandelion. Stain with corallin-soda.

From a potash maceration preparation tease out the laticiferous vessels and examine them.

Examine also the vessels in radial or tangential sections of the wood.

Examination of Ground Roasted Chicory

Procure some ground roasted chicory ; if in very coarse fragments, reduce these in a mortar to a finely granular powder.

Decolourise a few grains with solution of chlorinated soda and wash. Mount a little in dilute glycerin for examination. The parenchymatous cells and the reticulate or pitted vessels are very distinct, but the laticiferous vessels are not conspicuous ; they must be carefully looked for. Stain a portion of the decolourised powder with Soudan red ; the laticiferous vessels are now more easily seen, as their contents stain, more or less satisfactorily, red. Stain another preparation with tincture of

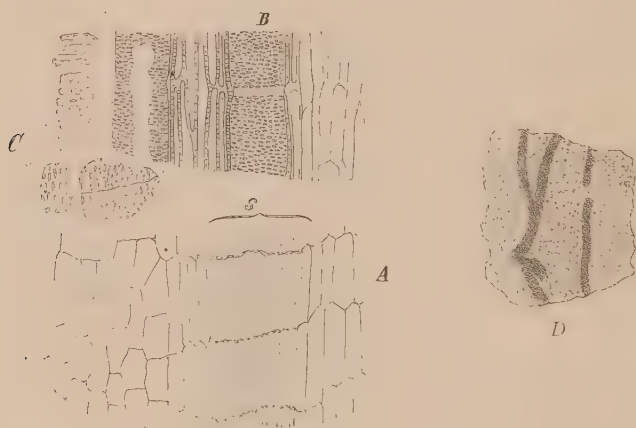


FIG. 163.—Chicory Root, fragments from the powder. *A*, portion of the cortex in longitudinal section, showing the sieve tubes, *s*, with sieve plates. *B*, portion of the wood in longitudinal section, showing the vessels ; $\times 70$. *C*, portions of the walls of the vessels ; $\times 240$. *D*, fragment of parenchyma of cortex with laticiferous vessels ; $\times 120$. (Schimper.)

alkanna diluted with an equal volume of water, allowing twenty-four hours for the absorption of the stain. This method sometimes affords better results than the Soudan red. Stain another portion with corallin-soda ; the callus plates stain pink, but fragments of sieve tissue staining with corallin are not very numerous in the powder.

Another portion of the ground chicory may be digested with solution of potash on a water-bath for fifteen minutes, washed, and examined in glycerin or chloral hydrate. This preparation often affords good results.

The following are the diagnostic characters of chicory root:

- (a) Abundant parenchymatous tissue in wood and cortex.
- (b) In the cortex laticiferous vessels and numerous small sieve tubes with transverse plates.
- (c) In the wood vessels of considerable size with large pits.

Ipecacuanha Root

Source.—The root of *Psychotria Ipecacuanha*, Stokes.

Preparation and Examination. Select several typical pieces of Brazilian ipecacuanha root, and soak them in water until sufficiently soft to cut. Cut transverse sections, and treat in the usual way.

Examine one in water; it contains abundance of starch; remove this by clearing in the usual way.

The tegumentary tissue consists of several rows of thin-walled flattened cells containing a brown granular substance to which the dark brown colour of the root is due; in surface view these cells exhibit an irregular polygonal outline.

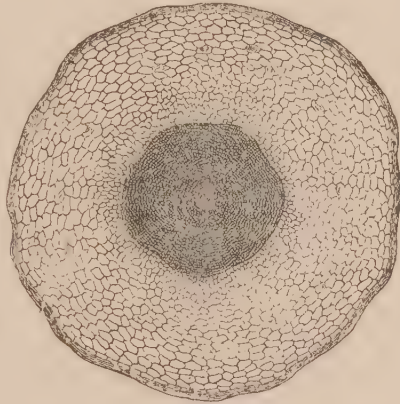


FIG. 164.—Ipecacuanha Root, transverse section, showing dense wood and large cortex. Magnified. (Planchon and Collin.)

The parenchymatous tissue that follows the cork is secondary cortex and consists of rounded or polygonal cells that are often axially elongated and exhibit few intercellular spaces. It passes without any visible line of demarcation into the bast ring, the bast itself forming wedge-shaped groups of cells, recognisable by their smaller size and by the difference in the cell walls. Here and there in the secondary cortex and bast are cells filled with what often appears to be a granular substance, but which on closer examination, especially of radial sections, proves to be acicular crystals of calcium oxalate.

The wood consists almost entirely of small elements, vessels not being distinguishable. The medullary rays are not con-

spicuous, all the cells of the wood showing, in transverse section, about the same radial elongation.

Stain a section, before clearing, with solution of iodine; more or less regular radial lines of cells, containing abundance of starch, can be detected. These cells probably correspond to and perform the function of medullary rays. A tangential

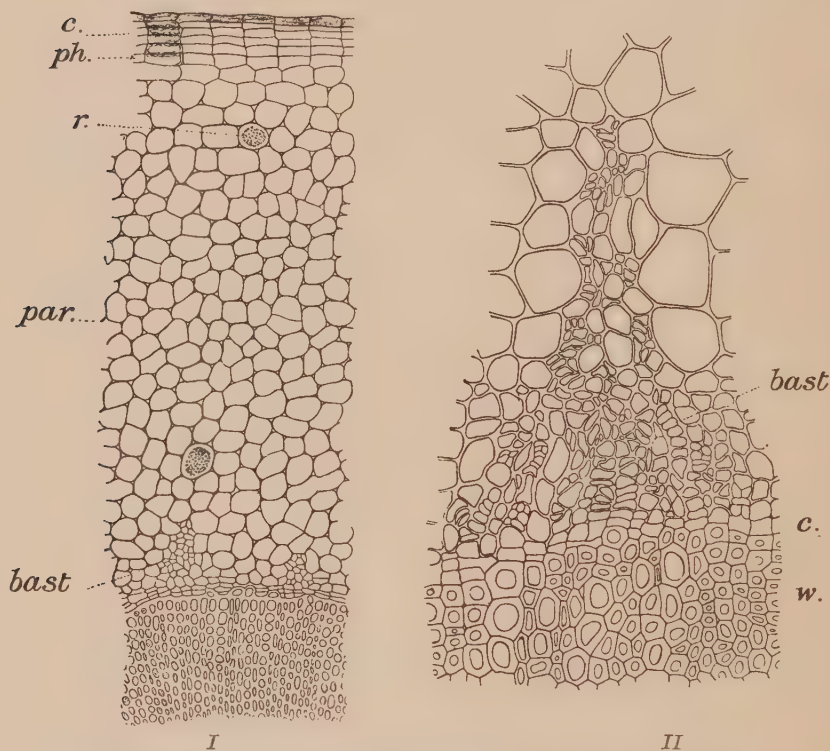


FIG. 165.—Ipecacuanha Root. I, transverse section of the cortex, with part of the wood; *c.*, cork; *par.*, parenchyma of cortex; *ph.*, phellogen; *r.*, raphides. II, portion of the same, more highly magnified; *c.*, cambium; *w.*, wood. (After Tschirch.)

section of the wood shows that they are strongly axially elongated and bear simple pits, but otherwise are not strikingly different from the other elements of the wood.

Macerate some fragments of the wood with potassium chlorate and nitric acid; examine the cells of which it consists. Vessels, at least typical vessels, are not to be found, but there are numerous tracheids and fibrous cells. The tracheids (fig. 166, *tr.*)

have more or less pointed ends and oblique transverse walls, in which a perforation may generally be detected. The pores are commonly oval, ascend in a left spiral, and are bordered. The fibrous cells have slit pores also arranged in a left spiral. They differ from ordinary wood fibres chiefly in the fact that they

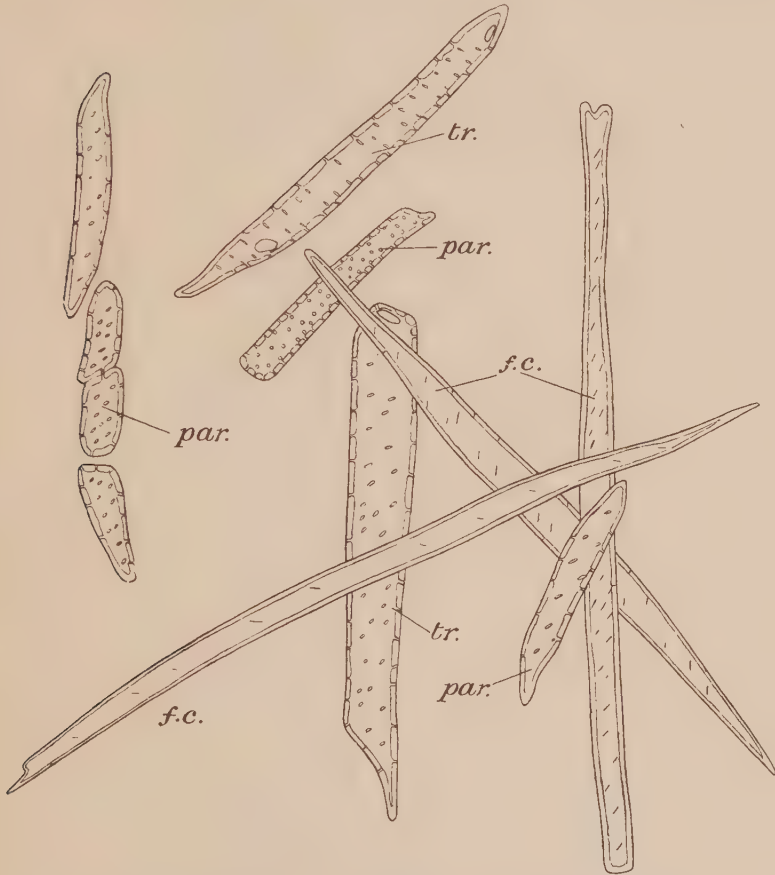


FIG. 166.—Brazilian Ipecacuanha, elements of root wood. *f.c.*, fibrous cells; *par.*, parenchymatous cells; *tr.*, perforated tracheids. $\times 250$.

contain starch, and hence are not solely mechanical in their function. In addition to these two forms of cells typical wood parenchyma (*par.*) may be found, as well as cells that are intermediate in character. True vessels are absent.

Examine next the starch grains; which can easily be separated by scraping the cut surface of a dry root. They are

mostly compound. The component grains number commonly from two to four, occasionally as many as eight or even more, and are most frequently muller-shaped, with one or two flat surfaces. The hilum is usually a distinct point, or its position may be indicated by a simple or triangular cleft. In length they occasionally reach $12.5\ \mu$, but never exceed $15\ \mu$.

Proceed next to examine the powdered drug. Mount a little in water or dilute glycerin, and examine the starch grains. Note the presence here and there of an acicular crystal of calcium oxalate and of fragments of parenchymatous cells, as

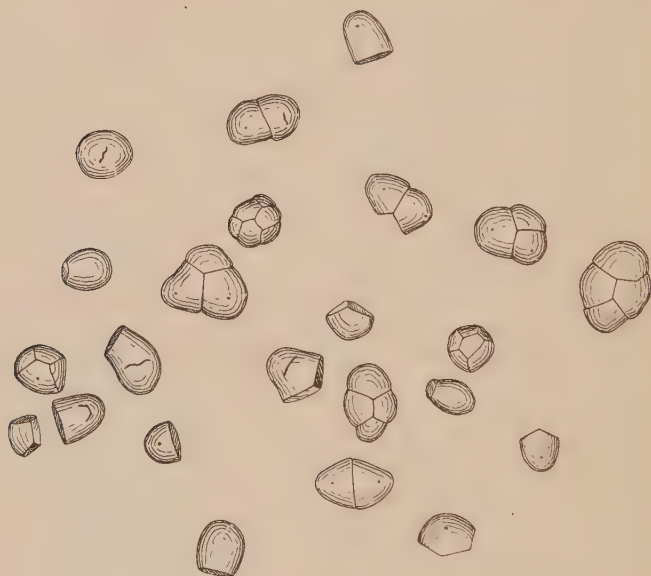


FIG. 167.—Starch of Brazilian Ipecacuanha Root. $\times 750$.

well as of wood. The details of the latter cannot well be identified without further preparation.

Mix about 0.5 gramme of the powder with 5 c.c. of water, and raise the mixture to the boiling-point, so as to gelatinise the starch; add 10 c.c. of nitric acid (1.42) and about 0.5 gramme of potassium chlorate. Warm gently for about five minutes, taking care that the action is not too vigorous. Dilute with about an equal volume of water, and separate by the centrifuge the tissues that have resisted destruction; wash once with water, separating by the centrifuge as before; then treat with a few cubic centimetres of chloral hydrate for a few

minutes, and separate again. Pour off the chloral hydrate solution, and examine the residue either in water, dilute glycerin, or chloral hydrate. No elements, especially no sclerenchy-

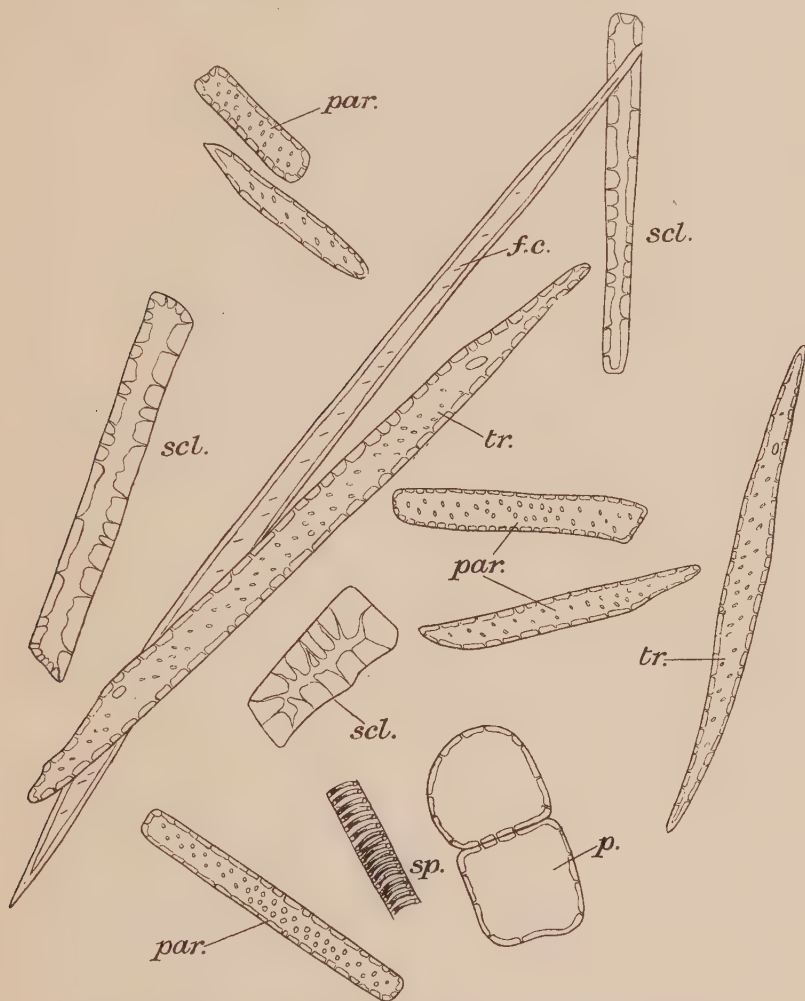


FIG. 168.—Brazilian Ipecacuanha, elements of stem wood. *f.c.*, fibrous cells; *p.*, lignified cells of pith; *par.*, parenchymatous cells; *scl.*, sclerenchymatous cells; *sp.*, spiral vessel. $\times 250$.

matous elements, but those previously found in the root should be present. The characteristic tracheids of the wood can be well seen and identified, but the perforations in the oblique

walls are sometimes difficult to find. Parenchyma that has escaped destruction is fairly abundant, and numerous fragments of cork may be found. The tracheids may be separated from one another by gently pressing upon and at the same time sliding the coverslip.

Ipecacuanha powder frequently contains a considerable proportion of the erect stem; this betrays itself (1) by the sclerenchymatous cells of the pericycle, (2) by the lignified cells of the pith, and (3) by the spiral vessels of the protoxylem.

Carthagena ipecacuanha, which has been substituted for the Brazilian root, is chiefly to be distinguished by its starch grains, which are, as a rule, larger than those of the Brazilian root. Large grains frequently attain 17 to 22 μ , the latter figure being rarely exceeded, whereas in the Brazilian root 15 μ is the maximum. It must, however, be observed that the size of the starch grains is somewhat variable, the maximum size being attained in the largest roots; hence, small roots of Carthagena ipecacuanha and large roots of the Brazilian drug contain starch grains of approximately the same size, and the powders derived from such roots cannot, therefore, be distinguished by this means.

On the other hand, the powder of any root that contains vessels in its wood can be easily detected by the presence of these in the sclerenchymatous tissue separated by treatment with potassium chlorate and hydrochloric acid.

APPENDIX A

REAGENTS OF GENERAL UTILITY

THE following list comprises the reagents mentioned in the foregoing pages, together with a few others that are occasionally employed. It makes, however, no pretensions to being exhaustive; on the contrary, numerous reagents that have been recommended from time to time for various special purposes, for which doubtless they are useful, have been omitted as being unnecessary for the student. This is especially the case with the host of staining reagents, of which but few have been retained as specially useful for particular purposes. Each reagent has been described, so that the student may either prepare it himself or easily procure it, and to most of them notes have been appended on the uses to which they are generally put. The student is strongly advised to refrain from the indiscriminate use more particularly of staining reagents. Each experiment should be designed to obtain certain definite information with regard to the preparation under examination.

Acetic Acid.—Containing 33 per cent. of real acetic acid; the Acid Aceticum of the British Pharmacopœia; it is used for distinguishing between calcium oxalate, which is insoluble, and calcium carbonate, which dissolves with effervescence, for neutralising an excess of caustic potash, and other purposes.

Acetic Acid, Dilute.—The Acidum Aceticum Dilutum of the British Pharmacopœia, containing 4 per cent. of real acetic acid.

Alcohol.—Absolute alcohol is to be preferred, but for many purposes methylated spirit made with wood naphtha can be used; the ordinary methylated spirit made with mineral naphtha is of limited use only, since it makes a turbid mixture with water.

Alcohol is employed for a great variety of purposes. It removes air from sections of dried drugs, dissolves resin, volatile oil, tannin, chlorophyll, &c.; it dissolves most fixed oils in small but appreciable quantity, castor oil freely. Gum and inulin are quite insoluble in it, certain sugars only slightly soluble.

Alcohol, 90 per cent., is also frequently used, and may be often employed in the place of absolute alcohol; it has less solvent action upon fixed oils.

Alkanna, Tincture of.—

Alkanet root	20 grammes.
Alcohol, 90 per cent.	100 cubic centimetres.

Macerate for a week, and filter.

Tincture of alkanna is much used as a staining agent for fixed oils. For this purpose it should be diluted with an equal volume of water immediately before use, and sections left immersed in it for several hours. No fixed oil or fat is known which will not, under these conditions, assume a red colour; but, on the other hand, other substances, such as resin, caoutchouc, &c., may also yield the reaction.

Ammonia, Dilute.—The *Liquor Ammoniaë* of the British Pharmacopœia, containing 10 per cent. by weight of ammonia gas.

Ammonia, Strong.—The *Liquor Ammoniaë Fortis* of the British Pharmacopœia, containing 32·5 per cent. by weight of ammonia gas. It is used for the preparation of cuoxam.

Aniline chloride.—A saturated solution in water is sometimes used to stain lignified cell walls, to which it imparts a golden yellow colour. It is, however, inferior in this respect to phloroglucin, and hence is seldom used. It acts better when acidified with hydrochloric acid.

Bismarck Brown.—A very dilute aqueous solution, about the colour of brown sherry, is useful to stain elements after separation by potassium chlorate and nitric acid, or by chromic acid, by which treatment they are rendered very transparent, and delicate details are difficult to see. (Compare also safranin.)

Braemer's Reagent.—

Sodium tungstate	1 gramme.
Sodium acetate	2 grammes.
Water to make	10 cubic centimetres.

Dissolve. This is one of the best reagents for tannin, with which it produces a yellowish-brown precipitate. While iron salts are commonly used to detect tannin, it must be remembered that a number of other plant constituents give dark greenish or bluish black colourations with this metal; hence, although a negative result is decisive, a positive result does not necessarily indicate tannin. In such cases confirmation is obtained by Braemer's reagent.

The section to be tested, which should not have been treated with any solvent of tannin, is immersed in a drop of the reagent on the

slide ; as the reagent penetrates a characteristic yellowish-brown or reddish-brown precipitate is produced.

Chloral Hydrate, Solution of.—

Chloral hydrate	50 grammes.
Water	20 cubic centimetres.

Dissolve. The solution is a most valuable clearing agent. It not only induces expansion of cells that have shrunk during the drying of the drug, but also dissolves many of the commoner constituents, such as chlorophyll, resin, volatile oil, proteid matter, starch, &c.

Chloral Iodine.—The foregoing solution saturated with iodine, a few crystals of which should be kept in it. It is useful for the detection of minute starch grains.

Chlorinated Soda, Solution of (Meyer).—

Chlorinated lime	200 grammes.
Distilled water	1750 grammes.

Triturate the chlorinated lime with the water, added gradually ; transfer to a stoppered bottle, and add—

Sodium carbonate	250 grammes,
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dissolved in

Distilled water	750 grammes.
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Shake together for four days, keeping the bottle protected from light ; filter ; to the filtrate add a 10 per cent. solution of potassium oxalate as long as a precipitate forms, stand, and filter.

Solution of chlorinated soda is extremely useful for bleaching sections and preparations the colour of which is too dark to allow of the details being clearly seen. The bleaching should not be continued longer than necessary ; when completed, the preparations should be washed with water.

The reagent should be kept protected from light.

Chlorzinciodine, Solution of.—

Solution of zinc chloride, sp. gr. 1·8	100 grammes.
Potassium iodide	10 grammes.
Iodine	0·15 gramme.

Dissolve the potassium iodide and the iodine in 10 cubic centimetres of water ; add this to the solution of zinc chloride ; stand until bright. Keep a few crystals of iodine in the solution.

The following (Squire's) method also yields good results :

Liq. zinci chloridi, B.P.	100 cubic centimetres.
Potassium iodide	10 grammes.
Iodine	0·2 gramme.

Evaporate the solution of zinc chloride to 70 cubic centimetres, dissolve the iodide of potassium in it and add the iodine; shake at intervals till saturated.

Very commonly used for the differentiation of cellulose from lignified walls. The section should be mounted in a drop of water on a slide, the water *completely* removed by filter paper, and a drop of the reagent dropped on to it. Cellulose walls are (often slowly) coloured blue or violet, lignified and suberised walls yellow or brown; starch grains swell, and are coloured blue.

Chromic Acid, Solution of.—

Chromic acid	10 grammes.
Dilute sulphuric acid (containing 10 to 15 per cent. of sulphuric acid) . . .	90 cubic centimetres.

Dissolve. The reagent is useful for separating sections into their constituent cells. Several sections are immersed in the reagent in a watch-glass, and one from time to time (about every fifteen minutes) removed, washed with a drop of water, and gently pressed with a glass rod. When, under this treatment, the constituent cells separate readily from one another, the remainder of the sections are washed with water and transferred to alcohol until required.

Long-continued action of the reagent results in the destruction and solution of the cellulose and lignified cell walls. Suberised walls resist its action much longer.

Corallin-Soda, Solution of.—

Sodium carbonate	30 grammes.
Distilled water	70 grammes.

Dissolve. To a little of this solution add a small fragment of corallin, or sufficient alcoholic solution of corallin to produce a bright pink colour. The mixture must be freshly prepared.

The special use to which corallin-soda is put is the staining of callus plates and the detection of sieve tubes by this means. The solution should be of a pale bright pink colour (not dark wine-red), and should be freshly prepared, as in this dilution it rapidly loses its staining power. The reagent also imparts a reddish colour to lignified tissue, starch grains, and some forms of mucilage.

Cuoxam.—This reagent must be freshly prepared with strong solution of ammonia. The following (Vogl's) method is a convenient one:

Prepare some cupric oxy carbonate by precipitating a solution of cupric sulphate with sodium carbonate; wash the precipitate first by decantation, afterwards on the filter until free from sulphate. Drain well and dry by exposure to the air. Keep the dry powder in a stoppered bottle. When the reagent is required, dissolve a little in strong solution of ammonia.

It dissolves cellulose.

Eosin.—A dilute solution in water is occasionally useful for staining cell contents; it colours proteid matter red.

Fehling's Solution.—

Cupric sulphate	34.6 grammes.
Distilled water to	500.0 cubic centimetres.

Dissolve.

Sodio-potassium tartrate	173.0 grammes.
Potassium hydrate	125.0 grammes.
Distilled water to	500.0 cubic centimetres.

Dissolve. Mix equal volumes of each solution for use when required. The reagent is used to detect reducing sugars, with which it yields a red precipitate of cuprous oxide; with some proteids bluish or reddish violet colouration is produced.

Ferric Chloride, Solution of.—A 1 per cent. solution of ferric chloride in distilled water. Frequently used as a reagent for tannin. See also Braemer's Reagent.

Glycerin.—Pure glycerin of sp.gr. 1.260.

Glycerin, Dilute.—Pure glycerin diluted with an equal volume of distilled water.

Both of these are very largely used as mounting media.

Gum and Glycerin.—

White gum acacia	40 grammes.
Water	30 cubic centimetres.
Glycerin	40 grammes.
Thymol	0.1 gramme.

Dissolve. Used for fixing small seeds, &c., on pith. The glycerin prevents the gum from being hard when dried.

Hydrochloric Acid.—Pure hydrochloric acid of sp.gr. 1.16. Used in conjunction with phloroglucin for detecting lignification. Diluted with an equal volume of water for dissolving calcium oxalate.

Iodine Water.—Distilled water saturated with iodine, a few crystals of which should be kept in the reagent. Protect it from the action of light by keeping it in the dark or in amber-coloured bottles. Used as a reagent for starch and for aleurone grains.

Iodopotassium Iodide, Solution of.—

Iodine	2 grammes.
Potassium iodide	1 gramme.
Distilled water	200 grammes.

Dissolve. The reagent, which may be diluted with water if

necessary, stains proteid matter yellow, starch blue, suberised and lignified walls yellow. The cellulose cell wall assumes a yellow colour, which is changed to blue by irrigation with concentrated sulphuric acid.

Maceration Mixture, Schulze's.—Potassium chlorate and nitric acid; the strength of the latter may be varied to suit the requirements of the case; an acid of sp.gr. 1·3 is very generally useful.

The reagent is well adapted for the separation of the elements of woody tissues from one another, and also for the destruction of the more delicate parenchymatous cells and their removal from powdered drugs.

Methylene Blue, Alcoholic Solution.—

Methylene Blue	0·1 gramme.
Alcohol (95 per cent.)	25·0 cubic centimetres.

Dissolve.

Methylene Blue, Glycerin Solution.—

Methylene Blue	0·2 gramme.
Alcohol (95 per cent.)	10·0 cubic centimetres.
Glycerin	40·0 cubic centimetres.

Dissolve. Useful for staining mucilage (compare p. 114).

Millon's Reagent.—

Mercury	3 cubic centimetres.
Fuming nitric acid	27 cubic centimetres.

Dissolve without heat; dilute the solution with an equal volume of water. Proteid matter in contact with Millon's reagent gradually assumes a bright brick-red colour. As the activity is liable to diminish by long keeping, it should be tested on a section known to contain proteid matter before it is used as a reagent.

Naphthol Solution.—

α -Naphthol	10 grammes.
Alcohol	100 cubic centimetres.

Dissolve. Gives in conjunction with sulphuric acid a violet colouration with inulin. Allow a drop of the reagent to remain on the section for a minute or two and remove with filter paper; drop on two or three drops of concentrated sulphuric acid, cover, and warm gently. An intense violet colouration is produced if inulin is present.

Osmic Acid, Solution of.—A 1 per cent. aqueous solution of osmic acid. It should be protected from light. Osmic acid gradually colours fixed oils dark brown or nearly black. It does not, however, react with all fats, palmitin, stearin, and certain others not being coloured by it.

Phloroglucin, Solution of.—

Phloroglucin	1 gramme.
Alcohol (90 per cent.)	100 cubic centimetres.

Dissolve. It gradually darkens in colour, and at the same time loses its power. It should not be kept more than three months. The section to be tested should be immersed in a few drops of the reagent for five minutes, the excess removed with filter paper, and a drop of strong hydrochloric acid added. Lignified cell walls are stained pale to dark red, according to the degree of lignification.

The reagent is sometimes made by dissolving phloroglucin in alcohol and adding hydrochloric acid.

Picric Acid.—A saturated aqueous solution is used to stain aleurone grains yellow.

Potash, Solution of.—A 5 per cent. aqueous solution of potassium hydrate. Largely used as a clearing agent. It induces swelling of the cell wall and consequent expansion of dried cells, swells and dissolves starch, dissolves proteid matter, tannin, &c. It is also employed for disintegrating parenchymatous tissues, these being digested in the reagent, diluted if necessary with water, in a water-bath.

Potash, Ammoniacal Solution of.—Wash stick potash with water to remove the carbonate on its surface, and add water in quantity insufficient to dissolve the whole of the potash. Pour off the saturated solution and add an equal volume of strong solution of ammonia (sp. gr. 0.910).

This reagent has been recently advocated as specially useful for the identification of fixed oils. It saponifies all fatty oils, producing with non-drying oils radiating filiform crystals, and with drying oils granules. The section is immersed in the reagent, covered with a coverslip, and examined from time to time, during several hours, to ascertain its effect upon the globules to be tested.

Potash, Very Dilute Solution of.—A 0.3 per cent. solution is used to dissolve aleurone grains.

Potash, Strong Solution of.—A 20 per cent. (or even 50 per cent.) solution is used to induce swelling of refractory cell walls with a view to disclosing the structure of collapsed tissues.

Ruthenium Red, Solution of (*in Solution of Lead Acetate*). Prepare a 10 per cent. solution of lead acetate in distilled water. To one or two cubic centimetres of this solution add enough ruthenium red to produce a wine-red colour. The solution will not keep long, and should therefore be freshly prepared.

A very useful reagent for the detection of mucilage, some varieties of which assume with it a brilliant pink colouration.

Safranin.—A very dilute aqueous solution is useful for staining colourless, transparent tissues to render the details more easily visible.

Soudan Glycerin.—

Soudan III.	0·01 grammes.
Alcohol (90 per cent.)	5·00 cubic centimetres

Dissolve and add—

Glycerin	5·00 cubic centimetres.
--------------------	-------------------------

Colours the suberised wall red, especially when warmed with it and hence is useful to detect secretion cells (the walls of which are commonly suberised) in powdered drugs. It also colours fixed and volatile oils.

Sulphovanadic Acid.—

Ammonium vanadate	1 gramme.
Concentrated sulphuric acid	100 cubic centimetres.

Powder the ammonium vanadate and triturate it with the sulphuric acid; stand until clear. The reagent will not keep longer than a few days. It is a delicate micro-chemical reagent for strychnine.

Sulphuric Acid, Concentrated.—Pure sulphuric acid of sp.gr. 1·843. It is employed for dissolving cellulose and lignified cell walls, leaving suberised walls comparatively little acted upon.

Sulphuric Acid, 80 per cent.—Sulphuric acid containing 80 per cent. by weight of the pure acid may often be advantageously substituted for the above.

APPENDIX B

LIST OF THE CHIEF VARIETIES OF CELL WALL AND CELL CONTENTS, AND THE MEANS ADOPTED FOR THEIR IDENTIFICATION

(1) Aleurone Grains.

- (a) Picric acid stains them bright yellow ;
- (b) Iodine stains them yellowish-brown ;
- (c) In iodine water the crystalloid and globoid (if present) become visible ;
- (d) In very dilute potash they dissolve, with the exception of the globoid and calcium oxalate (if present).

- (2) **Alkaloids.**—The best general reagent is solution of iodine in potassium iodide, which produces reddish-brown precipitates with almost all alkaloids, even in very dilute solutions. Sections that have been thus treated are compared with sections that have been freed from alkaloid by extraction with an alcoholic solution of tartaric acid before being submitted to the reagent. Special colour reactions, such as that of strychnine with sulphovanadic acid, often afford very valuable information. Further details must be sought in the numerous memoirs that have been published dealing specially with this subject.

(3) Calcium Carbonate.

- (a) Acetic or hydrochloric acid dissolves with effervescence ;
- (b) Sulphuric acid produces in addition acicular crystals of calcium sulphate.

(4) Calcium Oxalate.

- (a) Insoluble in acetic acid ;
- (b) Soluble without effervescence in hydrochloric acid ;
- (c) Yields acicular crystals of calcium sulphate with sulphuric acid.

(5) Callus Plate.

- (a) Corallin-soda stains bright pink ;
- (b) Hoffmann's blue stains blue ;
- (c) Sulphuric acid dissolves it.

(6) Caoutchouc.

- (a) Insoluble in caustic potash ;
- (b) Soluble in chloroform ;
- (c) Stains pink with tincture of alkanna.

(7) Cell Wall, Cellulose.

- (a) Is stained blue or violet with chlorzinciodine ;
- (b) Is stained blue with iodine followed by sulphuric acid¹;
- (c) Is not stained by aniline chloride or by phloroglucin ;
- (d) Dissolves in cuoxam.

(8) Cell Wall, Lignified.

- (a) Is stained yellow or brown with chlorzinciodine ;
- (b) Is stained bright yellow with aniline chloride ;
- (c) Is stained bright red with phloroglucin and hydrochloric acid ;
- (d) Swells and dissolves in strong sulphuric acid, especially if gently warmed.

(9) Cell Wall, Suberised.

- (a) Is stained yellow or brown with chlorzinciodine ;
- (b) Is stained red by Soudan red ;
- (c) Resists the action of concentrated sulphuric acid ;
- (d) Is stained yellow by strong potash ; on warming, oily drops exude.

(10) Fat.

- (a) In solid, often crystalline masses which fuse to oily drops when warmed ;
- (b) These are stained with tincture of alkanna ;
- (c) Is saponified by ammoniacal potash, producing crystalline or granular soaps ;
- (d) Is soluble in ether-alcohol.

(11) Inulin.

- (a) In colourless, amorphous, or sometimes sub-crystalline masses ;
- (b) Insoluble in cold water ;
- (c) Dissolves at once without swelling in water at 60-70° C.
- (d) Is not stained by iodine ;
- (e) Gives a violet colouration with α -naphthol and sulphuric acid.

(12) **Mucilage.**—Several varieties of mucilage are known which vary in their reactions ; the following are useful :

- (a) Insoluble in alcohol and glycerin ; swell and (?) dissolve in water ;
- (b) Solution of subacetate of lead colours them yellowish and makes them granular ;
- (c) May be stained by ruthenium red, corallin-soda, chlor-zinciodine, or methylene blue.

(13) **Oil, Fixed.**

- (a) In globules ;
- (b) Is stained pink with tincture of alkanna ;
- (c) Is stained brown with osmic acid ;
- (d) Is saponified by ammoniacal potash ;
- (e) Is soluble in ether-alcohol ; not readily soluble in 90 per cent. alcohol.

(14) **Oil, Volatile.**

- (a) In globules ;
- (b) Is stained red with tincture of alkanna ;
- (c) Does not yield soap with ammoniacal potash ;
- (d) Is soluble in 90 per cent. alcohol.

(15) **Proteid Matter.**

- (a) Is stained yellow or brown by solution of iodine ;
- (b) Is coloured red by Millon's reagent ;
- (c) Is coloured yellow by potash after nitric acid ;
- (d) Is coloured yellow by picric acid.

(16) **Resin.**

- (a) In irregular solid masses ;
- (b) Is stained red with tincture of alkanna ;
- (c) Is soluble in 90 per cent. alcohol.

(17) **Silica** (*if present as a visible cell content*).

- (a) Is unacted upon by any of the ordinary reagents ;
- (b) May be recognised unaltered in the ash after treatment with hydrochloric acid.

(18) **Starch.**

- (a) Is coloured blue by solution of iodine ;
- (b) Is swollen by caustic potash ;
- (c) Swells when boiled with water.

(19) **Tannin.**

- (a) Is coloured bluish-black or greenish-black with solution of ferric chloride ;
- (b) Gives brown or yellowish-brown precipitate with Braemer's reagent.

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